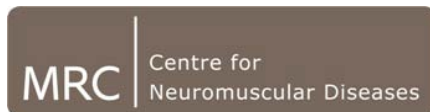


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# Welcome to the fourth annual meeting of the London Newcastle MRC Centre for Translational Research in Neuromuscular Diseases

## UK Neuromuscular Translational Research Conference 2011

Dear Colleagues,

We are delighted to welcome you to London for the fourth annual scientific meeting of the first MRC funded centre for translational research in neuromuscular diseases. We are very pleased that this annual UK Neuromuscular Translational Research Conference continues to be jointly hosted with the Muscular Dystrophy Campaign. This year we have worked closely with the MRC Mitochondrial Biology Unit, Cambridge and the Newcastle University Centre for Brain Ageing and Vitality to devise a dedicated translational mitochondrial session which is part of what we hope is an innovative and interesting overall programme. Other major themes this year include translational research in peripheral neuropathy "bench to bedside", the role of MRI as a diagnostic and monitoring tool in neuromuscular diseases and also a session on highlighting the lessons we can learn in applying findings in animal models to human diseases.

The MRC Centre for Neuromuscular Diseases aims to bring together clinicians, scientists, patient organisations and patients in order to advance UK translational research in neuromuscular diseases. This is a particularly exciting time in the field as a range of science discoveries are revealing an increasing number of therapeutic targets. The Centre aims to work with all its partners to support the development of a clinical trials culture for patients with neuromuscular diseases. We will continue to work hard to form effective research and clinical links with as many other UK neuromuscular groups as possible.

The MRC Centre was established in 2008 as a joint partnership between the UCL Institute of Neurology, Queen Square, the UCL Institute of Child Health and the University of Newcastle upon Tyne. The Centre is closely linked to its partner NHS organisations, University College London Hospitals NHS Foundation Trust, Great Ormond Street Hospital for Children NHS Trust and Newcastle upon Tyne Hospitals NHS Foundation Trust.

Over the next two days this conference aims to showcase a wide range of high quality scientific neuromuscular research from many UK groups, international colleagues and industry partners. There are focussed sessions on mitochondrial diseases, peripheral neuropathies, new MRI applications in neuromuscular disease and lessons from neuromuscular mouse models.

We have received over 90 high quality abstracts and there will be dedicated poster sessions each day as well as guided poster discussions. There will be four poster prizes for young investigators. All accepted abstracts are published in the journal *Neuromuscular Disorders*.

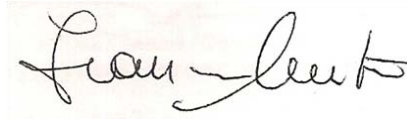
Professor Thomas Voit will deliver the second Victor Dubowitz Lecture, and Professor Robert Brown will deliver the second Morgan-Hughes-Thomas Lecture.

As the Director, I would very much like to thank the joint MRC-MDC meeting scientific planning team: Professors Katie Bushby, Doug Turnbull, Mary Reilly, Tarek Yousry, Dame Kay Davies, Sir John Walker, Volker Straub and Dr Marita Pohlschmidt. I also especially thank Zoë Scott and Julia Ambler for all their hard work in organising this meeting. Once again this annual meeting has been oversubscribed. We are very encouraged that there continues to be such strong interest in neuromuscular translational research from throughout the UK and beyond.

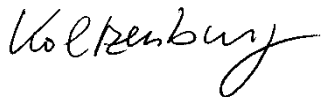
We sincerely hope that you have a stimulating and entertaining two days in London.



Professor Michael G Hanna  
Director  
MRC Centre for Neuromuscular Diseases,  
UCL Institute of Neurology



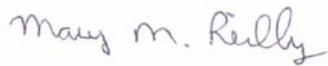
Professor Francesco Muntoni  
Deputy Director, ICH/GOS  
MRC Centre for Neuromuscular Diseases  
UCL Institute of Child Health



Professor Martin Koltzenburg  
Deputy Director, UCL/ION  
MRC Centre for Neuromuscular Diseases  
UCL Institute of Neurology



Professor Katie Bushby  
Deputy Director, Newcastle  
MRC Centre for Neuromuscular Diseases,  
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Professor Mary Reilly  
MRC Centre for Neuromuscular Diseases,  
UCL Institute of Neurology



Professor Dimitri Kullmann  
MRC Centre for Neuromuscular Diseases,  
UCL Institute of Neurology



Professor Doug Turnbull  
MRC Centre for Neuromuscular Diseases &  
Director, Newcastle University Centre for Brain  
Ageing and Vitality, University of Newcastle upon  
Tyne



Professor Volker Straub  
MRC Centre for Neuromuscular Diseases,  
University of Newcastle upon Tyne



Professor Tarek Yousry  
MRC Centre for Neuromuscular Diseases,  
UCL Institute of Neurology



Dr Marita Pohlschmidt  
Director of Research,  
Muscular Dystrophy Campaign



Professor Sir John Walker  
Director, MRC Mitochondrial Biology Unit,  
University of Cambridge



Professor Dame Kay E Davies  
Director, MRC Functional Genomics Unit  
University of Oxford

## **Welcome from Robert Meadowcroft – Chief Executive of the Muscular Dystrophy Campaign**

Welcome to the 2011 UK Neuromuscular Translational Research Conference organised in partnership between the MRC Centre for Neuromuscular Diseases and the Muscular Dystrophy Campaign.

This is the fourth time that the Muscular Dystrophy Campaign has been able to support this meeting and we are delighted, once again, that scientists and clinical researchers from across the field of neuromuscular disorders have an opportunity to showcase progress in the field with a particular spotlight on how these advances will translate into patient benefit.

The Muscular Dystrophy Campaign has supported research into neuromuscular disorders for over 50 years. During this time our families and supporters have raised more than £50 million to fund cutting-edge science and research, whilst a further £50 million has been invested in care and support for families. Despite the uncertain economic climate the charity is pressing forward and our partnership with Tesco raised over £4 million last year in support of much needed children's equipment.

The charity continues to successfully campaign for improvements in patient care and support by lobbying the Government and NHS decision makers to ensure patients with neuromuscular disorders can access specialist care. We are particularly pleased to have recently secured NHS funding for 19 Care Advisor posts across the UK – positions, that over the past 20 years, have been solely funded by the Muscular Dystrophy Campaign. As we all recognise, without a well resourced clinical infrastructure, treatments have no route out of the laboratory, so I would like to thank all our clinical colleagues who have worked so hard to help us make the case for a high quality national neuromuscular service.

This work is very much a team effort and the past 12 months has also seen 750 people living with muscle disease join forces with the Muscular Dystrophy Campaign to launch the National Muscle Group Support Network. The network consists of 12 individual 'Muscle Groups' that provide peer-to-peer support and secure new NHS investment and national media coverage. The groups are supported by over 100 MPs and 50 clinicians.

We are committed to building on these achievements as well as our research investment into neuromuscular disorders, and will continue to forge strong relationships with scientists and clinical researchers across the globe to ensure that emerging treatments have the best possible chance of leading to patient benefit as quickly as possible. It is a sad fact that time is a luxury these patients and families do not have.

Thank you for all the hard work that you have put into fighting muscle wasting disease over the last twelve months. I wish you well in your endeavours and hope that you have a very productive and enjoyable conference.



Mr Robert Meadowcroft  
Chief Executive, Muscular Dystrophy Campaign

## About the MRC Centre for Neuromuscular Diseases

Genetic and acquired neuromuscular diseases represent a major cause of mortality and morbidity in children and adults. In the UK there is a large gap between major science discoveries and patient benefit in these important disorders. This gap is larger in the UK than in other countries such as Germany, France and the USA who have already moved forward with translational research initiatives. The new MRC Centre aims to reduce this gap by establishing a multidisciplinary translational research activity in these disabling diseases.

This is a joint centre between the UCL Institute of Neurology and the UCL Institute of Child Health, London and the University of Newcastle. The Centre is building on long-established UCL-Newcastle research and clinical links. The centre is forming reciprocal clinical and research links with other neuromuscular research groups and patient organisations throughout the UK. The Centre works with the very large adult and paediatric neuromuscular disease patient populations cared for at the co-located hospitals: Great Ormond Street NHS Trust, the National Hospital for Neurology and Neurosurgery - Queen Square, UCLH NHS Foundation Trust and Newcastle Upon Tyne Hospitals NHS Foundation Trust.



Our mission is to translate basic science findings into clinical trials and new treatments for children and adults with disabling neuromuscular diseases. Current world-class science programmes in London and Newcastle attracting in excess of £20m of grant income will underpin the activities of the Centre. The Centre is developing new cross-cutting collaborations and has capitalised on the recruitment of world-class senior academic personnel to UCL and to the University of Newcastle. We have identified five key areas which we consider to be current obstacles to effective translation of basic science findings into patient benefit. These are: clinical trials support, availability of patient tissues and cells, assessing animal models, applying MRI to humans and animals and developing capacity for the future. The Centre is specifically addressing each of these obstacles.



- We are facilitating clinical trials in neuromuscular disease in the UK by forming a single clinical trials support activity drawing on and combining the expertise in London and Newcastle. We are taking advantage of the geography by forming north and south neuromuscular clinical trials centres. We are working together to facilitate clinical trial design, to develop biostatistical support, to develop clinical trial coordination, and to establish patient registries and clinician networks. We are taking advantage of well-established, government funded, collaborative specialist neuromuscular diagnostic services which already exist between London and Newcastle (NCG services). The MRC Centre is working closely with TREAT-NMD, the pan-European network of excellence, as the UK implementation partner.
- A shortage of human cell lines and neuromuscular tissues currently hinders basic science efforts and in vitro testing of potential therapies. We have now established a unique UK biobank of human neuromuscular patient tissues and cell lines for translational research.

- Assessing the validity of animal models of neuromuscular disease and correlating phenotypes with human disease remains an important problem. We are linking clinical and basic scientists, thereby establishing a network and resource for elucidating the validity of mouse models.
- We believe that the application of new MRI techniques has the potential to revolutionise the assessment and monitoring of neuromuscular disease in both animal models and patients. We are taking advantage of major new MRI facilities in London and Newcastle to establish cutting edge MRI of nerve and muscle disease in animals and humans.



- We recognise the critical importance of training the basic and clinical neuromuscular scientists of the future. The Centre has developed a brand new four-year neuromuscular disease PhD programme, and twelve science PhD students have now been appointed to this programme. We are ensuring that exciting translational research environments to train a new generation of basic and clinical neuromuscular scientists, building future capacity in the UK.

By developing these five core areas the Centre will promote translational research and add value to basic science neuromuscular research themes currently active in London, Newcastle and other centres.



## About the Muscular Dystrophy Campaign

The **Muscular Dystrophy Campaign** is the leading UK charity focusing on neuromuscular diseases. We have pioneered the search for treatments and cures for 50 years, and are dedicated to improving the lives of all children and adults affected by neuromuscular diseases.



We fund world-class research to find effective treatments and cures; provide practical and emotional support for individuals and families; campaign to raise awareness and bring about change, award grants towards the cost of specialist equipment, such as powered wheelchairs and provide specialist training and development for health professionals.

Since the Muscular Dystrophy Campaign was founded in 1959 we have supported scientists researching the underlying molecular basis of muscular

dystrophies and related neuromuscular conditions. In recent years, these investments have come to fruition and the focus of the research has begun to shift towards the development of therapeutic approaches.

We now need to invest in translational research - this is necessary because we need a speedy bench-to bedside transfer of promising technology. But this involves two-way interaction between the scientists and the clinicians. The basic bench science is important for understanding underlying causes of disease, something that can provide a plethora of potential drug or gene therapy targets.



Equally the observations that clinicians make at the bedside can provide a wealth of new information about a condition focussing the search for the scientist. There are however, many barriers in the meaningful progression of data and observations from the lab to something that ultimately will be life changing for people affected by these devastating conditions, and their families.

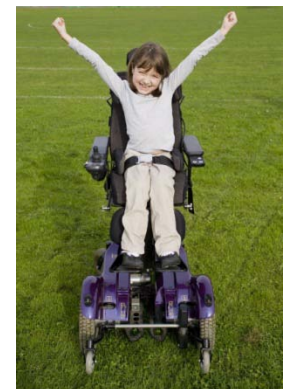


The Muscular Dystrophy Campaign aims to speed up this transition by providing support to both scientists and clinicians. We not only fund basic science through to pre-clinical research and where possible clinical trials. We also

provide logistic and financial support to create platforms where clinicians and scientists can meet, exchange experiences and discuss ideas.

One of our strategic aims is to fast-track promising treatment approaches when they are close to clinical trial and ensure rapid transition from bench-to-bedside. A primary focus of recent years has been to support and encourage initiatives to promote translational research in order to help remove some of the barriers faced by scientists and clinicians.

In order to give you an overview of the science that we support, we have invited our current grantees to present their work during the poster sessions. We hope these sessions will be of interest to you and will provide you with an update of the recent research advances that the scientists and clinical researchers have made.



## Patient Organisations

The MRC centre and the Muscular Dystrophy Campaign aim to develop strong links with all neuromuscular patient organisations.





## Programme

### UK Neuromuscular Translational Research Conference 2011

Kennedy Lecture Theatre, UCL Institute of Child Health, Guilford Street, London WC1N 1EH  
Tuesday 29<sup>th</sup> – Wednesday 30<sup>th</sup> March

#### Day 1 – Tuesday 29<sup>th</sup> March

- 09:00 – 10.15      Registration
- 10:15 – 10:30      Introduction**  
Professor Mike Hanna, UCL Institute of Neurology
- 10:30 – 12:30      Translational research in human mitochondrial diseases**  
**Jointly sponsored session MRC Centre for Neuromuscular Diseases, MRC Mitochondrial Biology Unit, University of Cambridge & Newcastle University Centre for Brain Ageing and Vitality**
- Chairs: Professor Sir John Walker, MRC Mitochondrial Biology Unit Cambridge, and Professor Patrick Chinnery, MRC Centre for Neuromuscular Diseases, Newcastle University**
- 10:30 – 11:00      Prevention of the transmission of mitochondrial diseases  
Professor Doug Turnbull, MRC Centre for Neuromuscular Diseases, Newcastle University
- 11:00 – 11:30      Developing therapies for human mitochondrial diseases  
Dr Michael Murphy, MRC Mitochondrial Biology Unit, University of Cambridge
- 11:30 – 12:00      Disturbance of calcium regulation and mitochondrial turnover as targets for therapy in mitochondrial disease  
Professor Michael Duchen, MRC Centre for Neuromuscular Diseases, University College London
- 12:00 – 12:15      Platform presentation  
The MRC Centre for Translational Research in Neuromuscular Disease: Mitochondrial Disease Patient Cohort Study UK  
Dr Robert Pitceathly, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology
- 12:15 – 12:30      Platform presentation  
Dominant and recessive *RRM2B* mutations cause familial PEO and multiple mtDNA deletions in muscle  
Dr Grainne Gorman, Newcastle University
- 12:30 – 13:30      The Second Victor Dubowitz Lecture**  
Introduced by Professor Francesco Muntoni
- 2011 State of the art in treatments for muscular dystrophy  
Professor Thomas Voit, Institute of Myology, Paris
- 13:30-14:30      Posters and Lunch

- 14:30 – 16:30**      **Peripheral neuropathy: bench to bedside**  
**Chairs: Professor Mary Reilly, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, and Professor Kristjan Jessen, UCL**
- 14:30 – 15:00      New molecular targets in hereditary neuropathies  
Professor Vincent Timmerman, University of Antwerp
- 15:00 – 15:30      Clinical trials in peripheral neuropathies – where have we got?  
Professor Richard Hughes, Cochrane Neuromuscular Disease Group
- 15:30 – 16:00      Animal models' contribution to the pathogenesis and treatment of inherited neuropathies  
Dr Michael Sereda, Max Planck Institute for Experimental Medicine, Göttingen
- 16:00 – 16:15      Platform presentation  
Neuregulin-1 is required for axoglial signalling following peripheral nerve injury to ensure normal re-myelination and functional recovery  
Florence Fricker, King's College London
- 16:15 – 16:30      Platform presentation  
Characterisation of novel mutations within heat shock protein 27 causing motor axonopathies  
Amy Innes, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology
- 16:30 – 17:00**      **Translational research at UCL and Newcastle**  
Professor Sir John Tooke  
Vice Provost (Health), Head of the UCL School of Life & Medical Sciences and Head of the UCL Medical School
- 17:00- 17:30      Posters & tea
- 17:30 – 18:30**      **The Second Morgan-Hughes-Thomas Lecture**  
Introduced by Professor Mary Reilly  
  
Motor Neuropathies - bench to bedside  
Professor Robert Brown, University of Massachusetts Medical School
- 18:30 – 19:30      Drinks reception & posters - Kennedy Lecture Theatre Foyer, ICH  
Introduced by Robert Meadowcroft, CEO, Muscular Dystrophy Campaign
- 20:00 - 22:45      Gala dinner - The Great Hall, BMA House, Tavistock Square  
Introduced by Professor Francesco Muntoni

**Day 2 – Wednesday 30<sup>th</sup> March**

- 09:00 – 11:30**      **New MRI applications in neuromuscular disease: a diagnostic and outcome measure tool in NM Disease?**  
  
**Chairs: Professor Tarek Yousry and Professor Volker Straub**  
**MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and Newcastle University**

09:00 – 09:30	Application of MRI in paediatric muscle disease Professor Eugenio Mercuri, Catholic University, Rome
09:30 – 10:00	Quantitative magnetic resonance imaging of neuromuscular diseases in adults Dr John Thornton, National Hospital for Neurology and Neurosurgery
10:00 – 10:30	Quantitative MRI in FSHD and DMD Dr Hermien Kan, Leiden University Medical Centre
10:30 – 11:00	MRI in LGMD2I Dr Tracey Willis, Newcastle University
11:00 – 11:15	Skeletal Muscle MRI-Determined Fat Fraction and Myometric Strength in Inclusion Body Myositis and Charcot-Marie-Tooth Disease Type 1A Dr Chris Sinclair, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology
11:15 – 11:30	Magnetic resonance imaging in the non-dystrophic myotonias Dr Jasper Morrow, MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology
<b>11:30- 13:15</b>	<b>Poster guided tours</b> Mitochondrial disease: Ian Holt & Shamima Rahman Peripheral neuropathies: Vincent Timmerman & Richard Hughes Neuromuscular MRI: Paul Matthews Neuromuscular animal models: Francesco Muntoni & Kay Davies Molecular therapies for DMD: Matthew Wood & Dominic Wells Congenital/myofibrillar myopathies & IBM: David Hilton-Jones & Janice Holton Muscle channelopathies & myasthenia gravis: David Beeson Muscle satellite cells: Jenny Morgan Neuromuscular databases: Adnan Manzur
13:15 – 14:00	Lunch
<b>14:00 – 16:00</b>	<b>Lessons from mouse models of muscular dystrophy and spinal muscular atrophy</b>  <b>Chairs: Professor Francesco Muntoni, MRC Centre for Neuromuscular Diseases, UCL Institute of Child Health, and Professor Dame Kay Davies, MRC Functional Genomics Unit, University of Oxford</b>
14:00 – 14:30	Outcome measures in the mdx mouse Professor Dominic Wells, Royal Veterinary College
14:30 – 15:00	Mouse models of SMA: implications for the timing and delivery of therapy Professor Kevin Talbot, University of Oxford
15:00 – 15:30	Novel insight in muscle and brain involvement in dystroglycanopathies Dr Susan Brown, Royal Veterinary College
15:30 – 16:00	A new mouse model of ALS carrying a point mutation in the mouse Sod1 gene Dr Peter Joyce, MRC Mammalian Genetics Unit, Harwell
<b>16:00 – 16:15</b>	<b>Poster prizes and close</b>

## Speaker Abstracts

### **Preventing transmission of mitochondrial disease**

Doug Turnbull, Mitochondrial Research Group, Newcastle University, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK & MRC Centre for Neuromuscular Diseases, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

Mitochondrial DNA disorders are caused by pathogenic mutations within the mitochondrial genome often leading to severe muscle disease in children and adults. Mitochondrial DNA is maternally transmitted and mothers with mitochondrial DNA mutations are at great risk of passing disease to their offspring. Maternally transmitted mitochondrial DNA mutations are either homoplasmic (with all copies mutated) or heteroplasmic (a mixture of both mutated and wild-type) and in the presence of heteroplasmy the severity of the clinical phenotype is directly related to the relative level of the mutated mitochondrial DNA. In the absence of curative treatment, preventing transmission of mitochondrial DNA mutations is a priority for families with inherited mitochondrial DNA mutations.

Recent research has shown that new IVF techniques could be used to prevent mitochondrial DNA mutations being passed from mother to child. Two papers have shown that both in primate and in human oocytes it is possible to change the mitochondria (including the mitochondrial DNA). This is done by swapping the nucleus from the oocyte of a woman with abnormal mitochondria and transferring it to a healthy donor oocyte of a woman with normal mitochondria, either before or immediately after fertilisation.

These studies hold great promise for families with mitochondrial DNA disease but there are many hurdles to overcome before this becomes a clinical treatment in the UK.

### **Developing therapies for human mitochondrial diseases**

Mike Murphy, MRC-Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK

Over the past few years myself and collaborators have developed antioxidants and nitric oxide donors that selectively block mitochondrial oxidative damage. Among these molecules are derivatives of the natural antioxidants ubiquinone and Vitamin E. The antioxidant efficacy of these molecules was increased considerably by targeting them to mitochondria, which are thought to be a major source of oxidative stress in mammalian cells. This was achieved by covalent attachment of the antioxidant to a lipophilic cation. Due to the large mitochondrial membrane potential, these cations accumulate several hundred fold within mitochondria, protecting them from oxidative damage far more effectively than untargeted antioxidants.

To see if this approach could be used to prevent mitochondrial oxidative damage in human diseases, we determined whether these compounds could be directed to mitochondria within mice. Non-toxic doses of mitochondrially targeted antioxidants could be fed to animals safely and led to the accumulation of intact antioxidant by mitochondria in the heart, skeletal muscle, liver and brain. The targeted version of ubiquinone (MitoQ) was protective against pathologies in animals. This molecule has since then gone through phase II trials as a potential therapeutic agent that may be associated with mitochondrial oxidative damage. I will report on progress towards this goal and also on the development of other related molecules which may also have potential as mitochondrial antioxidants and redox probes in investigating and treating mitochondrial diseases.

### **Dysregulation of calcium and mitochondrial function as potential therapeutic targets in muscle disease**

Michael R. Duchen, Katherine Heath, Neta B Baruch, Mike Hanna and Francesco Muntoni  
UCL MRC centre for Neuromuscular Diseases, UCL Consortium for Mitochondrial Research (C/MR) and Department of Cell and Developmental Biology, UCL, Gower Street, London WC1E 6BT, UK

Calcium signaling is fundamental to muscle excitation-contraction coupling, and so is integral to muscle physiology. Calcium signaling also has an intimate and multifaceted dialogue with mitochondrial biology. Calcium signals play a number of key roles in defining several aspects of

mitochondrial biology, while mitochondrial function influences the finer details of calcium signalling. Thus, cytosolic calcium signals associated with increased muscle activity or exercise promote mitochondrial biogenesis in muscle, operating through the PGC1 $\alpha$  transcription coactivator and AMPKinase (Ojuka et al., 2003). Calcium signals in the cytosol are transmitted to the mitochondrial matrix through the mitochondrial calcium uniporter. In the matrix, Ca<sup>2+</sup> upregulates the rate limiting enzymes of the TCA cycle and increases ATP synthase activity, increasing oxidative phosphorylation. Together, these mechanisms couple calcium signals associated with contraction, and so with increased energy demand, with increased ATP supply. Dysfunctional mitochondria may generate increased ROS. The Ca<sup>2+</sup> release probability of the Ryanodine Receptor (RyR) in skeletal muscle sarcoplasmic reticulum is regulated by vicinal thiol groups and is increased following oxidative modification (e.g. Stuart et al., 1992). Therefore altered mitochondrial function may readily alter the characteristics of the calcium signaling required to promote contraction. Further, mitochondria calcium import is dependent on the mitochondrial membrane potential and so if the potential is compromised (e.g. by an impaired respiratory chain), calcium uptake will be reduced. This may exacerbate and amplify a metabolic deficiency, as the calcium dependent regulation of oxidative phosphorylation will fail (e.g. see Brini et al., 1999). Finally, the accumulation of calcium by mitochondria means that they act as a spatial buffering system and so regulate the rates of propagation of calcium signals through the cell (Boitier et al., 1999).

It thus follows that disease that alters aspects of mitochondrial function (as in mitochondrial genetic disease) or that alters calcium signaling (as in diseases resulting from mutations of calcium signaling pathways) will have a major impact on these various mechanisms. In this talk I will review and illustrate these basic mechanisms and indicate potential points which seem to provide opportunities for therapeutic intervention in diseases that affect the mitochondrial calcium interface.

*We thank the Muscular Dystrophy Campaign, the Muscular Dystrophy Association and the BBSRC for financial support.*

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#### **The MRC Centre for Translational Research in Neuromuscular Disease: Mitochondrial Disease Patient Cohort Study UK**

Dr V Nesbitt,<sup>1</sup> Dr RDS Pitceathly,<sup>2</sup> Dr S Rahman,<sup>2,3</sup> Professor J Poulton,<sup>4</sup> Professor DM Turnbull,<sup>1</sup> Professor MG Hanna,<sup>2</sup> Dr R McFarland<sup>1</sup> <sup>1</sup>Mitochondrial Research Group, The Medical School, University of Newcastle upon Tyne, UK <sup>2</sup>MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London, UK <sup>3</sup>Mitochondrial Research Group, UCL Institute of Child Health, London, UK <sup>4</sup>Nuffield Dept Obstetrics and Gynaecology, Level 3 The Women's Centre, John Radcliffe Hospital, Oxford, UK

Mitochondrial disease is a debilitating and often life-threatening condition that affects at least 1 in 5000 people in the UK. A further 16.5 per 100000 people are at risk of developing the disease. The clinical phenotype is extremely variable and descriptions of the natural history are largely anecdotal with no systematic attempts to study mitochondrial disease progression in a tightly defined patient cohort.

The MRC Centre for Translational Research in Neuromuscular Diseases Mitochondrial Disease Patient Cohort Study UK has been established to create a national database of patients with biochemically or genetically confirmed mitochondrial disease, detail clinical phenotype, and correlate this with the underlying genotype. It is a collaborative project involving the three UK centres commissioned by



the Department of Health to provide clinical and diagnostic services for patients with mitochondrial disease based in Newcastle, London and Oxford. Development of both retrospective and prospective assessment tools will permit acquisition of accurate objective data on mitochondrial disease progression in both children and adults from the proposed cohort. This data will be crucial in terms of providing evidence based prognostic advice to patients and is a prerequisite for assessing efficacy of clinical interventions. The development of this cohort will facilitate large-scale interventional trials of drugs and novel treatments, and will also provide the opportunity to assess various prevention strategies including those for cardiomyopathy, stroke-like episodes, migraine and epilepsy. The unprecedented access to family data including genotyping will also permit definitive studies on the transmission of mitochondrial DNA (mtDNA) mutations and related to this, the effects of mitochondrial disease on female fertility and pregnancy.

### **Dominant and recessive *RRM2B* mutations cause familial PEO and multiple mtDNA deletions in muscle**

Carl Fratter<sup>1</sup>, Pravrutha Raman<sup>2</sup>, Charlotte L. Alston<sup>2</sup>, Emma L. Blakely<sup>2</sup>, Kate Craig<sup>2</sup>, Conrad Smith<sup>1</sup>, Julie Evans<sup>1</sup>, Anneke Seller<sup>1</sup>, Birgit Czermin<sup>3</sup>, Michael G. Hanna<sup>4</sup>, Joanna Poulton<sup>5</sup>, Charlotte Brierley<sup>6</sup>, Thomas G. Staunton MD<sup>7</sup>, Peter D. Turnpenny<sup>8</sup>, Andrew M. Schaefer<sup>2</sup>, Patrick F. Chinnery<sup>2</sup>, Rita Horvath<sup>2</sup>, Douglass M. Turnbull<sup>2</sup>, Grainne S. Gorman<sup>2</sup>, Robert W. Taylor<sup>2</sup>

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Progressive External Ophthalmoplegia (PEO) is a common manifestation of patients with mitochondrial myopathy, characterised by a progressive paralysis of the extraocular muscles leading to ptosis and ophthalmoparesis. The molecular genetic defect involves either primary mitochondrial DNA (mtDNA) mutations or mutations in nuclear-encoded mtDNA maintenance proteins leading to secondary mtDNA changes including multiple deletions. Mendelian forms of PEO are caused by mutations in five genes – *POLG*, *POLG2*, *SLC25A4*, *PEO1* and *OPA1*. A dominantly-inherited truncating mutation in a sixth gene - *RRM2B* - which encodes a subunit of the p53-inducible ribonucleotide reductase protein has recently been described in two families.

We have determined the frequency of *RRM2B* mutations in a cohort of 75 adult patients with PEO and multiple mtDNA deletions in muscle in whom mutations in known candidate genes had been excluded. Novel *RRM2B* variants were detected in 12 subjects (16% of patients). Ten patients with ptosis and ophthalmoparesis as their predominant clinical features harboured single, heterozygous changes; seven of these had dominant family histories and nonsense mutations in exon 9. Two patients with childhood-onset of symptoms harboured compound heterozygous, missense variants thus providing the first description of recessive *RRM2B* mutations associated with multiple mtDNA deletions. These cases displayed a variable spectrum of the clinical features of *RRM2B* mutations, which can show multisystem involvement when associated with infantile mtDNA depletion syndrome. Our data confirm *RRM2B* mutations to be a frequent cause of PEO and multiple mtDNA deletions, and that sequencing of this gene should be considered early in the diagnostic algorithm for multiple mtDNA deletion disorders.

### **New molecular targets in hereditary neuropathies**

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Most genes for Charcot-Marie-Tooth (CMT) disease and related hereditary peripheral neuropathies were identified through positional cloning or via a candidate gene approach. Different CMT phenotypes can be caused by mutations in the same gene, and conversely mutations in different genes may result in the same phenotype (i.e. genetic and phenotypic heterogeneity). This is further complicated by the fact that some mutations are private and occur in specific CMT subtypes.

Mutations in more than 20 genes cause primary alterations of the myelin sheath (demyelinating phenotypes). Well-known examples are myelin protein zero (MPZ), peripheral myelin protein 22 (PMP22) and connexin 32 (GJB1). Mutations in genes (e.g. neurofilament light chain, NFL) with a function in the axon however, result in axonal CMT phenotypes. Their gene products have cell type specific functions and therefore the underlying disease pathomechanisms can be logically inferred. Other mutations have been reported to cause an intermediate CMT, with both myelin and axonal phenotypes. More recently, CMT causing mutations were found in ubiquitously expressed genes coding e.g. amino-acyl tRNA synthetases, small heat shock proteins and enzymes involved in sphingolipid metabolism, where the resulting gene products have housekeeping functions and pleiotropic activities in many different cells and tissues. Therefore, these genes were not the obvious candidates for peripheral nerve degeneration and it remains an enigma why the mutant proteins cause such specific length-dependent degeneration of peripheral nerves. To find novel functional candidate genes, but also to identify peripheral nerve specific molecular pathways, we aim to pinpoint differential protein–protein interaction networks starting from the ubiquitously expressed genes (wild type versus mutant proteins). The identification of the interacting molecular partners and the higher-order molecular complexes they form part of will provide novel insights in regulatory pathways in health and disease, and contribute to new candidate genes for CMT neuropathies. Interacting proteins, and their encoding genes, are also potential candidates for other hereditary or sporadic peripheral neuropathies. Altogether, this will ultimately result in the identification of CMT gene networks which can provide novel insights in finding molecular targets for therapeutic intervention, not only for one type of CMT, but hopefully for several CMT subtypes, including the more rare and/or complex phenotypes.

### **Clinical trials in peripheral neuropathies – where have we got?**

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This review will contrast progress in the performance of clinical trials of diabetic, inflammatory and hereditary neuropathy. In diabetes mellitus, large trials of enhanced glycaemic control have shown a reduction in the incidence and severity of neuropathy in Type 1 and, recently, in type 2 disease. Based on the hypothesis that the sorbitol pathway is important in pathogenesis, aldose reductase inhibitors have been trialled but the results, summarised in a Cochrane review, have been negative. Other agents, notably alpha-lipoic acid, await Cochrane reviews and are still being tested. In chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), Cochrane reviews summarise the evidence from trials showing short term benefit from corticosteroids, plasma exchange and intravenous immunoglobulin. Trials of beta-interferon and methotrexate have been negative and sadly there are no ongoing trials of the many other candidate immunosuppressive agents. In Charcot-Marie-Tooth disease type 1a (CMT1a), the observation that ascorbic acid supplementation was beneficial in a mouse model has spawned at least 6 clinical trials which have yet to be summarised in a Cochrane review but the results are disappointing.

How can we do better? Trials in all three diseases require biometrically sound, clinically responsive outcome measures. Ideally these will be directly relevant to patients and health care providers and will allow definition of minimally clinically important, not just statistically significant, differences. In diabetes mellitus, the outcome of interest is amputation which does not occur until late in the disease so that surrogate measures are used. In CIDP, outcome measures have been extensively researched but only now is a responsive linear scale being developed, based on Rasch analysis. This requires comparison with conventional scales in a clinical trial setting. In CMT1a, a composite measure has been developed and shown to detect change, albeit only very slow, in adults. The scale is the best available but its complexity makes it difficult to interpret. In all three diseases the slowness of progression of the neuropathy requires the performance of long-term trials. The small amounts of change require large sample sizes. Except for diabetic neuropathy, the diseases are rare. These factors drive the need for multicentre and usually multinational trials. The sharing of outcome measures and times of observation between trials allows subsequent meta-analysis for which the CMT community is to be commended. Many of the trials which are most needed are not of interest to the pharmaceutical industry and alternative funding is difficult to find. A reduction in the

regulatory hurdles imposed by national and international regulatory authorities would greatly aid progress.

### **Contribution of animal models to the pathogenesis and treatment of inherited neuropathies**

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Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy, and a duplication of the PMP22 gene causes the most frequent subform, CMT1A. Transgenic rats (“CMT rats”) which harbour additional copies of Pmp22 phenocopy the pathology and clinical symptoms of CMT1A patients. CMT rats also develop a high disease variability. We have previously shown that lowering Pmp22 expression by anti-progesterone (Onapristone) treatment ameliorates the neuropathic phenotype in young CMT rats within weeks. We have extended this finding to adult CMT rats by starting treatment at a later time point in order to better mimic the clinical situation. Subcutaneous application of anti-progesterone for a period of 5 months allowed CMT rats to significantly gain increased muscle strength. Physical improvements can be fully explained by the prevention of axon loss. Surprisingly, the effects of anti-progesterone were not reflected by improved peripheral myelination, as measured by myelin sheath thickness. This demonstrates that the anti-progesterone therapy reduces Pmp22 overexpression to a degree at which the axonal support function of Schwann cells is better maintained than myelination. Thus, axonal loss is not caused by myelin pathology itself, but rather by a Schwann cell defect that has been partially uncoupled by anti-progesterone treatment. We are currently testing a number of drugs which target toxic Pmp22 overexpression and which may also convey neuroprotective properties in CMT. Pmp22 overexpression was also quantified in cutaneous nerves of CMT rats, and expression levels at young ages correlated with the clinical phenotype at later stages. We used the CMT rat to detect transcriptional markers which mirror acute disease severity and also prognostic markers of the individual future disease course. In a first attempt to translate these findings to the clinic we have validated disease marker expression in skin biopsies of CMT1A patients in order to establish sensitive biomarkers of disease severity and in particular disease progression. Experimental therapies and biomarkers derived from animal models should facilitate clinical trials in the future.

### **Neuregulin-1 is required for axoglial signalling following peripheral nerve injury to ensure normal re-myelination and functional recovery**

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Neuregulin-1 (NRG1) plays a crucial role in axoglial signalling during the development of the peripheral nervous system; its importance in adulthood following peripheral nerve injury remains unclear. We have generated an inducible NRG1-null mouse to suppress NRG1 expression in adulthood in order to avoid the confound of its developmental role. Following a sciatic nerve crush injury functional recovery as determined by measurement of the sciatic functional index (SFI) and re-myelination of axons visualised using electron microscopy was assessed in NRG1-null mice compared to vehicle and genetic controls.

Uninjured NRG1-null mice showed no functional deficit compared to controls. Axons and the associated myelin sheath were normal and there was no change in the total number of axons or Schwann cell (SC) nuclei. Following peripheral nerve injury NRG1 ablation resulted in a severe slowing in functional recovery, 17 days post injury control groups had returned to baseline on the SFI whereas at 42 days post injury NRG1-null mice had still not recovered to baseline levels. NRG1-null mice had severe defects in re-myelination; axons were either hypomyelinated or had no myelin sheath, this was not accompanied by a change in SC nuclei or total axons numbers. These findings indicate that Neuregulin-1 is necessary for normal re-myelination and functional recovery following peripheral nerve injury but is dispensable for maintenance of the myelin sheath in adult animals.

## **Characterisation of novel mutations within heat shock protein 27 causing motor axonopathies.**

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Heat shock protein 27 (Hsp27) is a well characterised small heat shock protein that plays a role in normal neuronal functions such as protein folding and degradation, axonal growth, and transport. Hsp27 also plays a role in cell survival, inhibiting apoptosis and protecting against oxidative stress.

Mutations in the Hsp27 gene were first shown to cause Charcot-Marie-Tooth Disease 2F/ distal Hereditary Motor Neuropathy II in 2004 by Timmerman's group and in 2008, we identified 3 novel mutations (Houlden et al, 2008). In the present study, we investigated the mechanisms that may underlie the pathological effects of these mutations. We replicated four mutations and examined the effects of transfection of neuronal-like SH-SY5Y cells with each mutation using wildtype Hsp27 as a control. In the first instance we examined mutational effects on cell survival, neurite outgrowth, cellular morphology and aggregate formation.

Every mutation increased the level of cell death under basal conditions, but only the S135F mutation enhanced vulnerability to cytoskeletal stressors Colchicine and Cytochalasin D. Although all mutations showed an increased disruption of cellular morphology, there was no discernible effect upon neurite outgrowth. However, one of the mutations presented nuclear aggregates in ~35% of all cells transfected and these aggregates were always found to co-localise with SC35, an mRNA splicing factor found in SC35-positive nuclear speckles.

These results demonstrate that all Hsp27 mutations examined have deleterious effects in neuronal cells. Furthermore, we have identified differential cellular effects caused by domain-specific mutations within the Hsp27 gene. Functional assays of virally-transduced motoneurons are now being used to elucidate the dysfunctional effects of these disease-causing mutations on the normal roles of Hsp27.

### **The Second Morgan-Hughes-Thomas Lecture**

Robert H. Brown, Jr., D.Phil., M.D., Professor and Chair, Department of Neurology, University of Massachusetts Medical School, Worcester, MA, USA

### **The Genetics of Motor Neurone Disease: from Molecules to Medicines**

Motor neurone diseases (also known as amyotrophic lateral sclerosis or ALS) is an adult-onset degenerative disorder of motor neurons, typically leading to paralysis and death in five years or less. About 10% of cases are inherited, usually as dominant traits (familial ALS or FALS). Over the last two decades, several FALS genes have been identified, including *SOD1*, *TDP43* and *FUS/TLS*. Numerous investigations support the view that the mutant FALS proteins are unstable and readily provoked to misfold, thereby acquiring toxic properties. Transgenic expression of mutant SOD1 protein in mice and cells generates animal and cell-based models of FALS, which have assisted in elucidating molecular events and targets for therapy. More recent data suggest that post-translational modifications of non-mutant SOD1 confer toxic attributes on the protein in sporadic ALS, mimicking the influence of the SOD1 mutations in FALS. These investigations have identified broad themes in the biology of motor neuron disease as well as approaches to therapy; these concepts are likely to be relevant to other neurodegenerative disorders.

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### **Quantitative magnetic resonance imaging of neuromuscular diseases in adults**

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**Introduction:** Magnetic resonance imaging (MRI) has revolutionized the diagnosis and management of many diseases, providing diagnostic sensitivity with technology which is non-invasive and well tolerated by patients. Careful acquisition design allows a move beyond conventional MRI to image-based measurements characterising the distribution and behaviour of tissue lipid and water, known to reflect underlying pathology. Potential benefits include improved diagnosis and a means to objectively monitor disease progression and therapeutic response in treatment trials. Current research aims to translate the established success of quantitative MRI (qMRI) of the brain and other organs to new applications in skeletal muscle and peripheral nerves.

**Methods:** We have investigated the applicability of qMRI in adults in a number of conditions affecting skeletal muscle and, or, peripheral nerves, including Charcot-Marie-Tooth disease type 1A, chronic idiopathic demyelinating polyradiculoneuropathy, inclusion body myositis and thyroid eye disease. Measurements have been performed across physical scales, from determination of organ size (peripheral nerve cross-sectional area) to measurement of the self-diffusion of water (DTI in skeletal muscle). Muscle magnetization transfer MRI and T2-relaxometry reflect the microscopic distribution and mobility of water molecules, and the chemical specificity of MRI enables quantification of muscle fat content, a known hallmark of neuromuscular pathology.

**Results:** Combining novel acquisition schemes with optimised conventional methods has allowed MRI quantification in clinically realistic examination times. Comparison between patient and healthy control groups and correlation with the best available clinical indices has demonstrated the ability of qMRI to provide clinically-relevant indicators of disease.

**Future Prospects:** Work will continue to advance qMRI applications across the spectrum of neuromuscular diseases, with a focus on achieving consistent inter-centre measures for multi-centre trials. A realistic aim is to deliver, within a timeframe of several years, new diagnostic tools to improve patient management and reliable imaging markers to enhance clinical trials of new treatments.

### **Quantitative MRI in FSHD and DMD**

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Muscular dystrophies like facioscapulohumeral dystrophy (FSHD) and duchenne muscular dystrophy (DMD) are characterized by progressive muscle weakness and loss of function. In the disease process, muscle tissue gets damaged, resulting in inflammation, fibrosis and replacement of muscle tissue with fat. Not all muscles are affected at the same time or at the same rate. In DMD, for instance, muscles in the proximal parts of the limbs are affected early in the disease, while the distal parts follow at a later stage. In FSHD, the facial and scapular muscles are affected first, while the leg muscles are affected later.

Due to these large differences in the rate and timing of affected muscles, a muscle biopsy will not always give a representative overview of tissue status. Alternatively, magnetic Resonance Imaging



(MRI) can provide a quantitative and non-invasive assessment of a large number of muscles at the same time. The main advantages of MRI over biopsy approaches are the excellent spatial resolution over a large imaging volume, and excellent soft tissue contrast without using ionizing radiation, thus allowing repetitive measurements. By using multiple MR-sequences, each sensitized to different aspects of tissue status, a multi-parameter description of tissue pathology can be obtained. So far, visual assessment of T1 weighted images are the clinical gold standard for assessment of muscle involvement in muscular dystrophies by scoring the level of fatty infiltration and muscle volume. However, to enable an accurate assessment of possible therapy effects and enable a comparison of scans from different sites, quantitative MR techniques are imperative. Recently, several of these techniques have been applied in FSHD and DMD. This presentation will provide an overview of the techniques used in these studies, and describe the patterns of muscle involvement that were uncovered using these techniques.

### **Assessing muscle pathology by MRI in LGMD2I**

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Limb Girdle Muscular Dystrophy 2I (LGMD2I) is caused by mutations in the fukutin related protein gene (FKRP) and associated with abnormal glycosylation of  $\alpha$ -dystroglycan.

In the past muscle MRI has proved useful in diagnostic purposes and more recently, has been identified as a potential non-invasive tool to monitor change.

This study aims to (i) assess fat infiltration patterns in a large, multi-centre cohort of LGMD2I patients using a qualitative radiological score on T1-weighted imaging (ii) to implement a 3-point Dixon technique for quantitative fat imaging, developing a non-invasive tool to track the progression of fat infiltration, and (iii) to correlate MRI findings with muscle strength and function.

(i) A total of 1140 muscles were analysed from qualitative T1w images. LGMD2I patients showed very few 22, (1.9%) normal (grade 0) muscles and 101, (8.9%) with mild early changes (grade 1), compared to 469 (41.1%) in the top 2 grades of abnormality. There is widespread involvement of all muscle groups, however some are better preserved until late stage. There are some subtle gender changes with sparing of the gracilis seen in the male subjects and the vastus medialis affected more in the male group compared to the female group.

(ii) A total of 1044 Regions of Interest were analysed on quantitative fat images and the amount of muscle involvement varied significantly from severe (median 70.1%), as in the biceps femoris (long head) to mild (median 5.9%) involvement in the tibialis anterior. The two techniques were highly correlated ( $r=0.813$  and  $p<0.0005$ ).

(iii) Good correlation was also seen between vastus medialis, a variably infiltrated muscle in both genders, and 6MWD, TUG and 10 metre run/walk which were  $r=-0.668$ ,  $r=-0.607$  and  $r=-0.690$  respectively and  $p<0.0005$ .

This study illustrates the usefulness of quantitative MRI as a potential outcome measure for muscular dystrophy trials.

### **Skeletal Muscle MRI-Determined Fat Fraction and Myometric Strength in Inclusion Body Myositis and Charcot-Marie-Tooth Disease Type 1A**

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Purpose: Fat infiltration of skeletal muscle is a common feature in both myopathies and peripheral neuropathies. We used the '3-Point Dixon' MRI fat-water quantification method to measure the tissue fat-fraction (FF) in the lower-leg muscles of patients with inclusion body myositis (IBM), Charcot-Marie-Tooth disease type 1A (CMT1A) and a control group. The relationship between FF and muscle function was investigated by correlation with myometry.

Methods: 16 IBM and 14 CMT1A patients and 16 healthy controls underwent 3-point Dixon MRI of the lower-leg at 3T and FF maps were generated. A radiologist placed regions of interest on the left and right tibialis anterior (TA) and soleus muscles of each subject and the mean FF for each region was recorded. Subjects underwent myometry with a CSMi HUMAC NORM Testing System. Maximum isometric ankle dorsiflexion and plantarflexion were recorded and compared with the TA and soleus muscle FFs respectively.

Results: Fat fractions were 3.1  $\pm$  4.7 %, 15.5  $\pm$  21.7 %, 16.7  $\pm$  27.2 % (mean  $\pm$  SD) in the control, IBM and CMT1A groups respectively. Muscle strength decreased with corresponding muscle FF in both patient groups [Spearman's  $\rho$  -0.53 ( $p=0.002$ ) and -0.65 ( $p<0.001$ ) for dorsiflexion, and -0.55 ( $p=0.001$ ) and -0.51 ( $p=0.005$ ) for plantarflexion in IBM and CMT1A respectively].

Discussion: MRI muscle FFs reflected fat infiltration in the patients, the large SDs reflecting disease heterogeneity. Future investigations, including correlations with isokinetic myometry will further elucidate the correspondence between MRI measures and muscle strength. Muscle FF may be a valuable marker of neuromuscular disease progression and treatment response.

### **Magnetic resonance imaging in the non-dystrophic myotonias**

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Objective: We aimed to assess the frequency and pattern of MRI abnormalities in patients with non-dystrophic myotonia (NDM). Methods: T1-weighted, T2-weighted and short-tau-inversion-recovery (STIR) sequences of the lower limbs of patients with genetically confirmed NDM. Images were analysed for T1-hyperintensity indicative of fatty infiltration and STIR hyperintensity indicative of oedema by a radiologist blinded to the diagnosis. Results: 11 patients with CLCN1 mutations and 10 patients with SCN4A mutations were enrolled with mean age 45.6, standard deviation 14.4 years, range 19-68 years. Fourteen patients were on medication for myotonia; 18 patients had normal strength on examination. All scans were abnormal. 20 patients had abnormal fatty infiltration, 19 patients had muscle oedema. Overall degree of fatty infiltration in thigh and calf were similar, except for patients with autosomal dominant myotonia congenita, where thigh muscles were less affected. The patterns of muscle involvement for fatty infiltration were similar in all groups with most involvement in the hamstrings in the thigh and relative sparing of posterior deep compartment muscles and lateral gastrocnemius in the calf. More oedema was seen in the calf muscles than the thigh muscles, especially medial and lateral gastrocnemius where STIR hyperintensity was seen in 76% and 57% of patients respectively. No correlation of MR-signal to age or medication use could be detected. Conclusion: MRI is abnormal in patients with non-dystrophic myotonias with both fatty infiltration and oedema in most patients despite normal power on examination.

### **Outcome measures in the *mdx* mouse**

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The *mdx* mouse has served as a vital biochemical and genetic model of Duchenne muscular dystrophy (DMD) since it was reported in 1984, with over 1800 articles citing the model in PubMed (January 2011). The *mdx* is a natural mutant that lacks dystrophin due to a premature stop mutation in exon 23 of the murine DMD gene. However, unlike patients with DMD who lose independent ambulation before their teens, the *mdx* mouse continues to be active throughout its life and does not show clear clinical signs of muscular dystrophy. Only one muscle, the diaphragm, appears to show the progressive fibrosis, loss of muscle fibres and fatty infiltrate that is characteristic of DMD muscles, although our recent work also shows fibre loss in limb muscles. Possible explanations for the difference between mouse and man range from differences in the forces exerted on the muscle fibre membrane to differences in the regenerative capacity of the

satellite cells. An alternative explanation is that muscle degeneration and repair do not scale with body size as lifespan does.

Many outcome measures have been used to demonstrate responses to various drug and gene therapy treatments in the mouse. Recent measures to set standard operating procedures (e.g. Grounds et al., *Neurobiol Dis.* 2008 Jul; 31(1): 1-19; <http://www.treat-nmd.eu/research/preclinical/dmd-sops/>) has done much to assist comparisons between labs and treatments, although different measures have differing degrees of reliability and interpretation. These outcome measures will be discussed and an argument presented that physiological measures in unconscious mice are the least ambiguous.

### **Mouse models of SMA: implications for the timing and delivery of therapy**

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Spinal muscular atrophy (SMA), caused by inactivating mutations in the survival motor neuron (*SMN*) gene, is characterized by loss of lower motor neurons in the spinal cord. The *SMN* gene and protein are very highly conserved in evolution, allowing the disease to be modeled in a range of species. The similarities in anatomy and physiology to the human neuromuscular system make the mouse the most suitable model for exploring the basic pathogenesis of motor neuron loss and for testing potential treatments. Therapies which increase SMN levels, either through direct viral delivery or by enhancing full length SMN protein expression from the *SMN1* paralogous gene, *SMN2*, are approaching the translational stage of development. It is therefore timely to consider the role of mouse models in addressing aspects of disease pathogenesis most relevant to SMA therapy. Evidence suggesting that the apparent selective vulnerability of motor neurons to SMN deficiency is relative rather than absolute, indicates that therapies will need to be delivered systemically. Analysis of neuromuscular junctions in mouse models also suggests that the SMN protein has its predominant action on the neuromuscular system in early postnatal life, during a discrete phase of development. Data from these experiments suggest that the timing of therapy to increase SMN levels may be critical. The extent to which SMN is required for the maintenance of motor neurons in later life and whether augmenting its levels could treat degenerative motor neuron diseases such as amyotrophic lateral sclerosis requires further exploration.

### **Novel insight in muscle and brain involvement in dystroglycanopathies.**

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Mutations in fukutin related protein (FKRP) are responsible for a common group of muscular dystrophies ranging from adult onset limb girdle muscular dystrophies to severe congenital forms with associated structural brain involvement, including Muscle Eye Brain Disease. The key defining feature of this group of disorders is the hypoglycosylation of  $\alpha$  dystroglycan and its inability to effectively bind various extracellular matrix ligands such as laminin  $\alpha$  2. In muscle this leads to basement membrane fragility that compromises the ability of the fibre to withstand repeated cycles of contraction and relaxation. However, the disease process may be more complex given that  $\alpha$  dystroglycan has the potential to interact with a number of laminin isoforms many of which are basement membrane/tissue specific and developmentally regulated. In order to investigate this aspect we used our FKRP knock-down mouse model (FKRP<sup>KD</sup>) to evaluate laminin  $\alpha$  chain expression in the cerebral cortex and eye. Our data show that in the cerebral cortex the hypoglycosylation of  $\alpha$  dystroglycan is associated with a marked disturbance in the deposition of several laminin  $\alpha$  chains including  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 4 and  $\alpha$ 5, although only laminin  $\alpha$  chain mRNA expression showed a significant up-regulation. The entire pial basement membrane appeared disrupted with a diffuse pattern of laminin deposition being evident below the pial surface. This pattern of deposition correlated with an abrupt termination of many of the radial glial cells which along with pial basement membrane defects contributed to the abnormal positioning of both early and late born neurons. In summary these observations demonstrate that a reduction in *Fkfp* influences the ability of  $\alpha$  dystroglycan to

direct the deposition of several different laminin isoforms. These findings should assist in the design of future therapeutic strategies for the FKRPs related group of diseases.

### **A new mouse model of ALS carrying a point mutation in the mouse *Sod1* gene**

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A potential drawback of current *SOD1* ALS mouse models is that they express mutant protein at higher levels than in ALS patients. Thus, some of the phenotypes described in these models could be caused by over-expression as opposed to the actual effects of mutant *SOD1*. We have used a chemical mutagenesis approach to create a more physiologically relevant model of ALS to try and overcome the disadvantages that underlie the *SOD1* transgenic models. We screened the Harwell N-ethyl-N-nitrosourea (ENU) archive, containing 10,000 matching DNA and sperm samples, for mutations in the *Sod1* gene. ENU is a potent mutagen that causes genome-wide point mutations. We have identified an allelic series of ENU induced mutants carrying mutations in the mouse *Sod1* gene: D83G, E109G and R115H. All three mutated residues are mutated in ALS cases, with the D83G mutation carrying the same amino acid change. Using an array of behavioural, physiological and histological techniques, we have begun to characterise *Sod1* D83G homozygote and heterozygote mutant animals. Initial behavioural phenotyping of homozygote mice has revealed ALS-like defects such as hind-limb tremor onset, abnormal gait, and decreased grip strength, body weight and startle response. Importantly, *Sod1* D83G homozygote mice generate less muscle force and show a reduced number of lumbar motor neurons at 15 weeks. The identification of this *Sod1* D83G ENU derived mouse provides the ALS community with a new model of disease, which will be a powerful tool for analysing early stages of disease.

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## Poster Abstracts

### DMD – Molecular Therapy

#### Poster 1

##### **Quantification of exon skipping in Duchenne muscular dystrophy by qRT-PCR**

Karen Anthony, Jennifer Morgan, Francesco Muntoni

*Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK*

Antisense oligonucleotide (AON)-mediated exon skipping of the dystrophin gene has emerged as a promising therapeutic intervention for Duchenne muscular dystrophy (DMD) which is currently undergoing phase II clinical trials. AON efficacy is traditionally first assessed by nested RT-PCR due to the low abundance of dystrophin. However, semi-quantitative end point detection is extremely variable, time consuming and relies on imprecise size discrimination. A quantitative assessment of dystrophin exon skipping has not been performed in any of the published studies. Here we describe the development of a Taqman qRT-PCR assay for skipped and unskipped dystrophin targets and highlight its use to relatively determine the percentage of exon skipping in DMD patients treated intra-muscularly with AON AVI-4658. This valuable assay could be used for the systematic selection of lead AON sequences, and allows for the accurate assessment of AON efficiency and the stratification of the rate of response in different patients recruited into clinical trials. Furthermore, applications for qRT-PCR across the wider field of the dystrophinopathies include the determination of dystrophin transcript levels between Becker muscular dystrophy patients with the same deletion but different levels of protein, adding an exciting new contribution to existing efforts to quantify protein levels in these patients.

#### Poster 2

##### **Human skeletal-muscle derived CD133+ cells as a promising tool for cell therapy of Duchenne muscular dystrophy**

Rowan Asfahani, Jinhong Meng, Francesco Muntoni and Jennifer Morgan

*Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

Duchenne Muscular Dystrophy (DMD) is an x-linked disease resulting in the degeneration of skeletal muscles due to the lack of dystrophin protein. Stem cell therapy is considered a promising strategy for the treatment of DMD; however, factors such as the type of stem cell, experimental animal models used for pre-clinical testing and the routes of transplantation have great impacts on transplantation efficiency. Human skeletal muscle-derived CD133+ cells have been reported to be able to give rise to a large amount of donor muscle after both intra-muscular and intra-arterial transplantation, thus suggesting them as a promising candidate for future stem cell therapy. However, application of CD133+ cells is limited by their extreme rarity within human muscles, as well as difficulties in isolating and expanding them *in vitro*. Here, we report that we have been able to isolate CD133+ cells from skeletal muscle by magnetic cell sorting and expand and maintain them *in vitro*. Cells were phenotypically similar and able to differentiate into myotubes and give rise to reserve satellite cells after expansion in three different media *in vitro*. In addition, these CD133+ cells gave rise to muscle fibres and satellite cells of donor origin following intra-muscular grafting into tibialis anterior muscles of immunodeficient *c5-/rag2-/ $\gamma$*  chain- mice. Therefore, we confirmed and extended the previous findings that human muscle derived CD133+ cells could be a promising stem cell candidate for the future clinical application for DMD.

#### Poster 3

##### **Exon skipping and dystrophin restoration in Duchenne Muscular Dystrophy patients after systemic phosphorodiamidate morpholino oligomer treatment**

Sebahattin Cirak MD<sup>1</sup>, Virginia Arechavala-Gomez PhD<sup>1</sup>, Michela Guglieri MD<sup>2</sup>, Lucy Feng PhD<sup>1</sup>, Silvia Torelli PhD<sup>1</sup>, Karen Anthony PhD<sup>1</sup>, Maria Elena Garralda MD<sup>4</sup>, Dominic J Wells VetMB PhD<sup>5</sup>, George Dickson PhD<sup>6</sup>, Matthew JA Wood MD PhD<sup>7</sup>, Steve D Wilton PhD<sup>8</sup>, Volker Straub MD<sup>2</sup>, Stephen B Shrewsbury MD<sup>9</sup>, Caroline Sewry PhD<sup>1,10</sup>, Jennifer E Morgan PhD<sup>1</sup>, Kate Bushby MD<sup>2</sup>, Francesco

Muntoni MD<sup>1</sup> <sup>1</sup>The Dubowitz Neuromuscular Centre, UCL Institute of Child Health London, UK; <sup>2</sup>Institute of Human Genetics, Newcastle University, Newcastle, UK; <sup>3</sup>DNA Laboratory Genetics Centre, Guy's Hospital, London, UK; <sup>4</sup>Academic Unit of Child and Adolescent Psychiatry, Imperial College St Mary's Campus, London UK; <sup>5</sup>Royal Veterinary College, London, <sup>6</sup>Royal Holloway University of London, UK; <sup>7</sup>Department of Physiology, Anatomy and Genetics, University of Oxford, UK; <sup>8</sup>Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Perth; <sup>9</sup>AVI BioPharma, Bothell, WA, USA; <sup>10</sup>Centre for Inherited Neuromuscular Disorders, Robert Jones and Agnes Hunt NHS Trust, Oswestry, UK

**Objective:** We report biochemical efficacy and clinical safety of a phase 1b/2 dose-ranging study of IV administered AVI-4658, an antisense morpholino oligomer which restores the reading frame and enables dystrophin expression in Duchenne muscular dystrophy (DMD) patients with amenable deletions for exon 51 skipping.

**Method:** AVI-4658 was given weekly for 12 weeks by intravenous infusions in a dose escalation study (0.5, 1.0, 2.0, 4.0, 10 and 20mg/kg) to 19 ambulant DMD patients aged 5-15 years. The primary study objective was safety and tolerability over the 26 week study. Secondary objectives were: 1) pharmacokinetic properties, 2) ability of AVI-4658 to induce exon skipping and dystrophin expression by RT-PCR, immunohistochemistry and immunoblotting.

**Results:** AVI-4658 was well tolerated with no drug related serious adverse events. We compared dystrophin levels in pre- and post-treatment (two weeks after last dose) muscle biopsies. AVI-4658 induced exon 51 skipping in the first 4 cohorts, followed by both stronger skipping and dystrophin protein expression in a generally dose dependent, but variable, manner in boys from cohort 3 onwards. A total of 7 subjects displayed an increase of dystrophin expression in the post-treatment biopsy, with 3 subjects showing a substantial increase in positive fibers (15%, 21% and 55%, respectively). Additionally dystrophin associated proteins were restored at the sarcolemma. Analysis of the inflammatory infiltrate indicated a reduction of cytotoxic T cells in the post-treatment muscle biopsies in the 2 higher dose cohorts.

**Conclusion:** We demonstrate the potential of AVI-4658 to become a disease modifying drug in the treatment of DMD.

#### Poster 4

##### Correction of FKRP function via RNA trans-splicing

Sarah Farmer<sup>1</sup>, Stephanie Lorain<sup>2</sup>, Adrian Thrasher<sup>3</sup>, Luis Garcia<sup>2</sup>, Francesco Muntoni<sup>1</sup>, Francesco Conti<sup>1\*</sup> <sup>1</sup>Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guildford Street, London WC1N 1EH, UK <sup>2</sup> Institut National de Santé et de Recherche Médicale, Groupe Myologie, Université Pierre et Marie Curie, Paris, France. <sup>3</sup> Molecular Immunology Unit, UCL Institute of Child Health, 30 Guildford Street, London WC1N 3BG, UK.

Mutations in fukutin-related protein (FKRP), which are common in the Caucasian population, cause loss of sugar coating in dystroglycan and lead to several forms of muscular dystrophy, ranging from limb girdle muscular dystrophy to more severe congenital variants such as Walker Warburg Syndrome. No effective treatment exists for these conditions, characterised by deterioration of muscle and heart function and eventual early death of affected individuals. One approach to treatment is to restore the function of the protein in muscle by correcting mutations in the FKRP gene. To this end we aim to employ a novel technology, RNA trans-splicing, which has shown promising results in preclinical models of diseases such as cystic fibrosis and Duchenne muscular dystrophy. In this form of gene therapy, a faulty exon within an endogenous transcript is targeted for trans-splicing-mediated replacement, which conveys the advantages of reduced transgene size and, importantly, retention of the endogenous regulation and expression pattern.

We are currently testing a panel of pre-trans-splicing molecule (PTM) constructs in order to optimise the trans-splicing event with an exogenous FKRP minigene in a wild type cell line. Following selection of the most effective PTM and validation of the approach, we aim to apply the treatment to cell lines derived from patients and, subsequently, to FKRP-mutant mice to determine effectiveness *in vivo*. Ultimately, RNA trans-splicing could represent a novel form of therapy for patients with FKRP mutations and, appropriately modified, for patients with other mutations that are not easily corrected via conventional gene therapy.

## Poster 5

### **Utrophin Upregulation in DMD Therapy: Current Status and New Tools for the Future**

Rebecca J. Fairclough†, Dave Powell†, Sarah Squire†, Allyson Potter†, Akshay Bareja†, Angela J. Russell\*¶, Steve G. Davies\* and Kay E. Davies† *tMRC Functional Genomics Unit, University of Oxford, Department of Physiology Anatomy and Genetics, South Parks Road, OX1 3QX* \*Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA ¶Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT

Duchenne muscular dystrophy (DMD) is a devastating X-linked muscle-wasting disease caused by lack of the cytoskeletal protein dystrophin. There is currently no effective treatment. By pharmacologically upregulating the dystrophin-related protein utrophin, we aim to develop a therapy applicable to all DMD patients by targeting the primary defect and restoring sarcolemmal stability in DMD. BMN-195 (SMTC1100) - the lead compound identified from our recent small compound screening programme in collaboration with Summit plc - recently become the first drug for utrophin upregulation to enter human Phase I trials. Poor pharmacokinetics led to trial termination and the drug is being re-formulated. It is vital that new drugs are now identified. We have developed improved *in vitro* and *in vivo* screening tools for this purpose. Compounds with novel targets in the utrophin promoter will be identified using an immortalised myoblast cell line developed from the new utrophin luciferase (*LUMdx*) knock-in mouse model. This will mimic the *in vivo* situation and enable us to identify compounds which transcriptionally upregulate utrophin through key elements recently identified outside of the 10 kb promoter A fragment that formed the basis of our previous screen. By enabling quantification of drug efficacy *in vivo* without having to sacrifice the mouse and terminate the trial, the new *LUMdx* model will enable us to assess drug efficacy and optimise timing and delivery of the drug without sacrificing the mouse. This approach will dramatically increase our capacity to perform high-throughput *in vivo* trials for utrophin upregulation in the *mdx* mouse.

## Poster 6

### **AAV-U7snRNA mediated multi exon-skipping for Duchenne Muscular Dystrophy**

Aurélie Goyenville<sup>1</sup>, Arran Babbs<sup>1</sup>, Jordan Wright<sup>1</sup>, Luis Garcia<sup>2</sup> and Kay E. Davies<sup>1</sup>

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Most cases of Duchenne muscular dystrophy (DMD) are caused by mutations that disrupt the dystrophin mRNA reading frame. In many cases, skipping of a single exon could restore the reading frame, giving rise to a shorter but still functional quasi-dystrophin protein. It has previously been proposed to use small nuclear RNAs, especially U7snRNA, to shuttle antisense sequences designed to mask key elements involved in the splicing of targeted exons.

Our present project focuses on the optimisation of U7snRNA constructs to complete rescue of dystrophin by exon-skipping in DMD patients. In particular, we are investigating the multi exon-skipping of exons 45 to 55, which could rescue up to 63% of DMD patients with a deletion.

In order to achieve this multi-skipping, we have first developed U7snRNA constructs targeting every exon between 45 and 55. Each construct has been inserted into lentiviral vectors for *in vitro* analysis in myoblasts from DMD patients. After transduction of these cells with lentiviral vectors encoding the various U7 constructs, specific skipping of the targeted exon was confirmed by RT-PCR. In parallel, we demonstrated the efficacy of these constructs *in vivo* in transgenic mice carrying the entire human DMD locus (*hDMD* mice) after intramuscular injection of AAV vectors encoding the U7snRNAs. Based on *in vitro* and *in vivo* results, the U7snRNA constructs inducing the most efficient skipping for each targeted exon were selected and combined into multi-skipping vectors. These AAV vectors are currently being tested in DMD myoblasts and in *hDMD* mice and efficient skipping of up to six exons has been demonstrated thus far.

These very encouraging results provide evidence that efficient multi exon-skipping can be achieved using AAV vectors encoding multiple U7snRNAs. These new constructs offer therefore very promising tools for clinical treatment of DMD.

## Poster 7

### **The feasibility of exon skipping to restore the reading frame in DMD patients with duplications**

Jihee Kim, Karen Anthony, Victoria Cloke, Michael Yau, Steve Abbs, Jennifer E Morgan, Francesco Muntoni, *Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London WC1N 1EH, UK*

Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, leading to disruption of its reading frame. Exon skipping trials, using antisense oligonucleotides (AONs) in DMD boys with out-of-frame deletions (targeting exon 51) have shown promising results. AON could theoretically be beneficial for patients with out-of-frame duplications (15% of all DMD mutations).

However, the application of AONs to duplicated patients faces unique challenges. Firstly, it is not known what is the genomic configuration of the duplicated regions is (tandem or not); secondly AONs not only recognise duplicated exons, but also normal exons, leading to complex patterns of skipping. Furthermore, double skipping to remove duplicated regions could be problematic in patients with large duplications. Finally efficient skipping of whole duplicated exons may lead to produce unstable proteins.

We firstly characterised the genomic configuration of the duplications, using both transcription studies from skeletal muscle or muscle cell cultures and Comparative Genomic Hybridization (CGH) arrays.

Secondly we evaluated the splicing patterns in patients with duplications to assess the feasibility of exon skipping strategies to restore the disrupted reading frame in cultured myoblasts.

Our results show that there was a good correlation between CGH array result and transcription studies except in one case. In cultures of selected duplicated patients, AONs induced multiple skipping of duplicated exons, and this was accompanied by the production of dystrophin protein as detected by western blotting, suggesting that this approach could be viable in at least some of the DMD with duplications.

## Poster 8

### **Optimal dystrophin mini-construct for gene delivery to skeletal muscle**

Mojgan Reza<sup>1</sup>, Steve Laval<sup>1</sup>, Jenny Morgan<sup>2</sup>, Francesco Muntoni<sup>2</sup>, Kate Bushby<sup>1</sup>, Volker Straub<sup>1</sup> and Hanns Lochmüller<sup>1</sup> <sup>1</sup>*Institute of Human Genetics, University of Newcastle upon Tyne, UK*  
<sup>2</sup>*UCL Institute of Child Health, 30 Guildford Street, London WC1N 1EH, UK*

Dystrophin is the largest known human gene and contains 79 exons, covering 2.5 million base pairs. Its cDNA sequence is 14 KB, coding for a 427 KDa protein. The large size of the dystrophin cDNA makes gene transfer strategies to treat DMD by transferring of the missing dystrophin into muscle fibers difficult due to the packaging limit of traditional viral vectors. The main focus of this research is to design and develop an optimal truncated dystrophin construct and determine which parts of dystrophin can be removed without resulting in a functional loss. Using overlap extension PCR, we have designed a number of different dystrophin truncated forms, encoding the various components of the dystrophin molecule. The constructs will be assessed conducting several *in vivo* and *in vitro* tests for functionality, such as membrane stability following osmotic shock as measured by creatine kinase release in immorto-mouse myoblasts and *in vivo* electroporation into mdx muscle. After injection we will observe if the specific force in mdx muscles is improved and whether there is a difference in contraction-induced injury in mini-dystrophin treated mdx mice. This initial assessment will determine the overall suitability of the different constructs for cloning into lentiviral vectors. We are aware of challenges to gene transfer approaches and know that further long-term experiments are required to assess the potential of this strategy but this project may bring us a few steps closer to a rapid testing system and a full systemic pre-clinical trial in *mdx* mice.



## Poster 9

### **Patient-specific Viral Rescue Therapies for Duchenne Muscular Dystrophy**

Morten Ritso<sup>1</sup>, Emily Dick<sup>2</sup>, Mojgan Reza<sup>1</sup>, Steve Laval<sup>1</sup>, Volker Straub<sup>1</sup>, Chris Denning<sup>2</sup> and Hanns Lochmüller<sup>1</sup> <sup>1</sup>*Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK*  
<sup>2</sup>*Wolfson Centre for Stem Cells, Tissue Engineering & Modelling (STEM), Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK*

Due to the exceptionally large size of the dystrophin gene and the various mutations that give rise to this condition, development of a ubiquitous rescue therapy for Duchenne Muscular Dystrophy (DMD) is extremely difficult. There have been various clinical trials that target post-translational processing of the dystrophin gene including exon skipping and premature stop codon read-through. However, considering the overall diversity of the genetic causes these treatments would only be applicable in a fraction of DMD patients.

Recent developments in the field of Adeno-associated viruses (AAV) have led to a sophisticated organ-targeting mechanism where serotype affinity has been employed. Micro-dystrophin-containing AAVs have been successfully used for transduction of cardiac cells. This method has been shown to alleviate the symptoms of DMD in mouse models and provides a promising area for further investigation. Moreover, considering the restricted insert size for AAV-mediated gene therapy, it may be more appropriate to introduce larger variants of shortened dystrophin with lentiviruses. The optimisation of the use of these prospective vectors will form the core for determining the most suitable and successful viral rescue therapy.

Standard procedures for deriving Induced Pluripotent Stem Cells (iPSC) have been employed for in vitro creation of patient-specific cells for rescue therapies in various conditions. Looking into how patient specific iPSC-derived cardiomyocytes respond to this proposed therapy allows a more objective in vitro assessment for potential improvements and eventual clinical trials. This interdisciplinary research can give hope to a larger cohort of DMD patients.

## Poster 10

### **Identifying genomic pre-clinical biomarkers for diagnostics and therapeutics of Duchenne Muscular Dystrophy**

Irina Zaharieva<sup>1</sup>, Sebahattin Cirak<sup>1</sup>, Jennifer Morgan<sup>1</sup>, Francesco Muntoni<sup>1</sup> <sup>1</sup>*Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N1EH, UK*

Duchenne Muscular Dystrophy (DMD) is a X-linked inherited disorder caused by mutations in the dystrophin gene. It affects one in 3500 males and leads to progressive muscle degeneration, loss of ambulation and death. Despite having mutations in a common gene, individual patients respond differently to drug treatment, suggesting that other factors such as modifying genes play a role in determining the phenotype. Better genetic characterisation of patients with DMD will provide valuable information about the variants influencing the disease severity and these variants could be used as biomarkers. Biomarkers in DMD are of great importance as they can benefit accurate diagnosis, monitoring of disease progression and assessing therapeutic outcomes. In recent years, methods for whole exome and transcriptome sequencing have been developed and such innovative technologies facilitate the identification of DNA or RNA differences for genetic biomarker discovery. As both DNA and RNA analysis will be performed on the same patients, a potential cause for different gene expression levels can be confirmed at the DNA level. The validation of the identified variants will prompt their usage as prognostic, pharmacodynamic and therapeutic biomarkers.

## Animal Models of Neuromuscular Disease

## Poster 11

### **A reduction in the expression of Fukutin-related protein leads to the altered deposition of multiple laminin alpha chains in a mouse model for Muscle Eye Brain Disease.**

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Glycosylated alpha dystroglycan (ADG) is an essential component of the dystrophin-associated glycoprotein complex (DGC) and functions as a bridge between the cell membrane and the extracellular matrix (ECM). Previously reported ECM ligands of ADG include laminin alpha 2, perlecan, agrin and neurexin which bind to glycosylated epitopes on ADG. Loss of these glycans, specifically that identified by I1H6, is associated with a group of neuromuscular disorders collectively termed the dystroglycanopathies. Fukutin related protein (FKRP) is one of six determined/putative enzymes implicated in the glycosylation of ADG.

We have previously generated a FKRP knock-down mouse which displays a hypoglycosylation of ADG and reduced laminin alpha 2 immunostaining at the muscle sarcolemma, the pial basement membrane and the inner limiting membrane (ILM) of the eye. Of particular interest is the absence of either I1H6 or laminin alpha 2 at the ILM in control or FKRP<sup>KD</sup> mice indicating that a novel ADG axis is involved at this basement membrane. In this paper we present evidence of an altered deposition of several laminin alpha chains in the eye and brain and suggest that multiple ligand interactions contribute to the pathogenesis of the dystroglycanopathies. Furthermore we have evidence indicating that DG interacts with laminin alpha 1 in the eye and the brain and that levels of laminin alpha 1 gene expression are up-regulated as a result of FKRP mediated hypoglycosylation of ADG.

### Poster 12

#### **Generation of a new mouse model for therapeutic testing in the dystroglycanopathies.**

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Mutations in fukutin related protein (FKRP) are responsible for a common group of muscular dystrophies ranging from adult onset limb girdle muscular dystrophies to severe congenital forms with associated structural brain involvement, including Muscle Eye Brain Disease. A common feature of these disorders is a variable reduction in the glycosylation of skeletal muscle  $\alpha$ -dystroglycan (ADG). Glycosylated ADG is an essential component of the dystrophin-related glycoprotein complex (DGC) whose known ECM ligands include laminin alpha 2, perlecan, agrin and neurexin. The glycans that decorate ADG mediate these interactions and their loss, specifically those identified by the antibody I1H6, are associated with a group of neuromuscular disorders now collectively known as the dystroglycanopathies. We have previously generated a mouse with a knock-down in Fkfp expression levels (FKRP<sup>KD</sup>) due to insertion of a floxed neomycin cassette in intron 2 of the mouse *Fkfp* gene. Since this mouse dies at birth due to central nervous involvement we have now replaced FKRP activity in the developing neural tube by crossing this line with one expressing Cre recombinase under the Sox-1 promoter. This has resulted in a near normal lifespan associated however with a clear muscle phenotype by 12 weeks of age. This mouse should prove invaluable in the design and testing of future therapeutic strategies in the dystroglycanopathies.

### Poster 13

#### **Zebrafish Fukutin family proteins link the unfolded protein response with dystroglycanopathies**

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Allelic mutations in putative glycosyltransferase genes, *fukutin* and *fukutin-related protein (fkrp)*, lead to a wide range of muscular dystrophies associated with hypoglycosylation of  $\alpha$ -Dystroglycan, commonly referred to as dystroglycanopathies. Defective glycosylation affecting Dystroglycan-ligand interactions is considered to underlie the disease pathogenesis. We have modelled dystroglycanopathies in zebrafish using a novel loss-of-function *dystroglycan* allele and by inhibition of Fukutin family protein activities. We show that muscle pathology in embryos lacking Fukutin or FKRP is different from loss of Dystroglycan. In addition to hypoglycosylated  $\alpha$ -Dystroglycan, loss of

Fukutin or FKRPP causes notochord defects and perturbs expression of laminins before muscle degeneration. These are a consequence of ER stress and activation of the unfolded protein response (UPR), preceding loss of Dystroglycan-ligand interactions. Together, our results suggest that Fukutin family proteins may play important roles in protein secretion and that the UPR may contribute to the phenotypic spectrum of some dystroglycanopathies in humans.

#### Poster 14

##### **Generating Stable FKRPP Mutant Zebrafish Lines with Zinc Finger Nucleases**

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Fukutin related protein (FKRPP) is a putative glycosyltransferase involved in the glycosylation of  $\alpha$ -dystroglycan. Deficiency of FKRPP in humans can result in conditions ranging from milder limb girdle muscular dystrophy to more severe congenital muscular dystrophy. Currently there are no stable mutant zebrafish lines for FKRPP deficiency. The best model system available is the transient knockdown of FKRPP expression by anti-sense morpholino (MO) injection into zebrafish embryos. This system gives a range of phenotypes but the knockdown has limited duration. A stable mutant line would negate the problems of a transient knockdown system which include decrease of knockdown efficiency due to cell division and consequent dilution of MO with time. Zinc Finger Nucleases (ZFNs) which are artificial nucleases specific to 9 base pairs (bp) target regions either side of a 5-7bp spacer. ZFNs are capable of causing a range of genomic mutations at the target site by inducing double-strand break repair through non-homologous end joining. There are two strategies being adopted, these are Oligomerized Pool Engineering (OPEN) and Context Dependent Assembly (CODA). The sequences generated in each approach for the zinc fingers are cloned into a vector containing the Fok-I nuclease domain. RNA from the expression vectors is injected into the embryo at the single cell stage. Mutant embryos will be screened by genotype against database sequences for mutations. Immunostaining will be used to investigate the phenotypes of the mutants focussing on eyes and muscle.

#### Poster 15

##### **Deposition of the Inner Limiting Membrane in the Eye of a Mouse Model for Muscle Eye Brain Disease**

Whitmore, Charlotte, Ackroyd, Mark R., Ashraf, Attia and Brown, Susan C. *The Royal Veterinary College, London NW1 0TU, UK*

Dystroglycan is composed of  $\beta$ -dystroglycan (transmembrane) and  $\alpha$ -dystroglycan (peripheral membrane protein), which contains the laminin binding site. The dystroglycanopathies are a group of muscular dystrophies characterised by the hypoglycosylation of  $\alpha$ -dystroglycan. Several forms such as Walker Warburg Syndrome and Muscle Eye Brain disease are associated with eye abnormalities. To further investigate this we have used a mouse model of Muscle Eye Brain disease which displays a reduction in fukutin related protein (Fkrpp) levels and defective basement membrane deposition.

We have previously demonstrated that the eyes of our newborn FKRPP knock-down mice show breaches in the ILM, but glycosylated  $\alpha$ -dystroglycan is not a component of this basement membrane at this time point, even in wild type mice. We have now investigated this further by examining the pattern of  $\alpha$ -dystroglycan glycosylation and associated basement membrane proteins at the ILM from E12.5. Additionally, we show here the ILM defects seen in the FKRPP knock-down mouse are associated with defects in retinal structure and lamination.

#### Poster 16

##### **Investigating pathophysiology and therapeutic strategies in a mouse model of spinal and bulbar muscular atrophy (SBMA)**

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Spinal and bulbar muscular atrophy (SBMA) is a late onset motor neuron disease (MND) caused by a polyglutamine (CAG) repeat expansion in exon 1 of the androgen receptor (AR) gene on the X-chromosome. Although the protein is widely dispersed, mutant AR results in selective motoneuron loss in the spinal cord and brain stem nuclei. The disease presents predominantly in adult males and is characterised by weakness, atrophy and fasciculations of facial, bulbar and proximal limb muscles.

In this study we investigated a yeast artificial chromosome (YAC) transgenic mouse model of SBMA with 100 CAG repeats (AR100) in the N terminal of the human androgen receptor gene (Sopher *et al*, 2004). The mice develop a late onset neuromuscular phenotype with accompanying lower motoneuron degeneration. The AR100 mice therefore recapitulate many of the phenotypic and pathological characteristics of the human disease.

In a previous study, we established that pharmacological upregulation of the endogenous Heat Shock Response (HSR) significantly improves disease progression in the SOD1 mouse model of Amyotrophic Lateral Sclerosis (ALS), a rapidly progressing motoneuron disorder (Kieran *et al*, 2004; Kalmar *et al*, 2009). We have therefore examined the possibility that upregulation of the HSR may also have beneficial effects in SBMA mice. Our results show that upregulation of the HSR in SBMA mice improves disease phenotype when treatment is started after disease onset. In view of these positive findings, we are currently investigating the effects of presymptomatic treatment since it is likely that this strategy may be even more effective than that observed following postsymptomatic treatment. Such an approach is relevant for SBMA as pre-symptomatic individuals who carry the mutation can be genetically identified.

#### Poster 17

##### ***In Vivo* Myocardial Calcium Influx is Increased in the Delta sarcoglycan Deficient Mouse Model of Muscular Dystrophy Cardiomyopathy. Role Of The L-Type Calcium Channel.**

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Intracellular calcium is increased in muscular dystrophy cardiomyopathy, although it is unclear whether this is due to ion channel defects or sarcolemmal injury. Mn<sup>2+</sup> which enters cardiac myocytes through L-type calcium channels can be used to monitor *in vivo* myocardial calcium influx using MRI as it is a contrast agent on T1 weighted images. Delta sarcoglycan deficient mice (model of LGMD2F), mdx mice (model of DMD) and wild type C57BL/10 mice were studied using MRI. Sarcolemmal injury was determined with Evan's Blue Dye fluorescence microscopy. Mdx and DSG KO mice had left ventricular hypertrophy and preserved left ventricular systolic function, DSG KO mice also had increased heart rates. Mn<sup>2+</sup> contrast enhancement was elevated in DSG KO mice and borderline elevated in mdx mice. The L-type calcium channel blocker diltiazem (5mg/kg ip) reduced Mn<sup>2+</sup> contrast enhancement in WT and mdx mice but not in DSG KO mice. However high dose diltiazem (10mg/kg ip) did reduce contrast enhancement in DSG KO mice. Likewise, heart rate reduction in response to diltiazem was impaired in DSG KO mice though not in WT or mdx mice. Mdx mice had increased levels of sarcolemmal injury, though this was only borderline increased in DSG KO mice. In DSG KO mice, both increased *in vivo* myocardial calcium influx and heart rates are relatively resistant to L-type calcium channel blockade. In contrast, in mdx mice sarcolemmal injury appears more important. These results demonstrate that different muscular dystrophy cardiomyopathies have heterogeneous underlying pathophysiology, with important implications for potential therapies.

#### Poster 18

##### **Investigating novel mutant mouse models of motor neuron disease**

Philip McGoldrick<sup>1</sup>, James Dick<sup>1</sup>, Tom Ricketts\*, Peter Joyce\*, Pietro Fratta<sup>1</sup>, Abraham Acevedo-Arozena\*, Elizabeth Fisher<sup>1</sup> & Linda Greensmith<sup>1</sup> <sup>1</sup>*MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*, and \**MRC Harwell*

Mutations in TAR DNA binding protein (TDP-43) have been identified as causes of sporadic and familial motor neuron disease (MND). TDP-43 is a ubiquitous, multifunctional protein, crucially involved numerous roles including gene expression and RNA metabolism. Despite identification of many disease-related mutations, it remains unclear as to why mutations in the protein cause motoneuron degeneration.

We have identified two lines of mice from the ENU (ethylnitrosourea) chemical mutagenesis program at MRC Harwell, with a single point mutation (Q101STOP, K160R) in endogenous mouse TDP-43. Thus the mutant proteins are at physiological levels and recapitulate the protein-protein interactions found in humans. These mice are being investigated with a combination of *in vitro* and *in vivo* techniques.

In the first instance we examined embryonic motoneurons for evidence of cellular pathology. Since TDP-43 normally plays a role in neurite outgrowth and stress granule (SG) formation, we assessed these characteristics in primary embryonic motoneurons. Results showed no deficit in neurite outgrowth in mutant TDP-43 motoneurons, although these cells formed significantly fewer SGs than wildtype motoneurons on exposure to an oxidative stressor, suggesting that the mutant effects of TDP-43 may manifest following cellular stress

Examination of neuromuscular function in aged mutant TDP-43 mice *in vivo* showed that mutant TDP-43 mice do not display deficits in hindlimb muscle force, muscle contractile characteristics or motor unit survival. Following the *in vitro* findings of deficits in SG formation, we are currently examining whether the effects of mutant TDP-43 may only manifest *in vivo* under conditions of cellular stress such as that which occurs following peripheral nerve injury.

#### **Poster 19**

##### **Transient mouse models for the preclinical evaluation of therapeutic dystrophin exon skipping strategies**

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Antisense oligomer (AO)-mediated splicing manipulation can remove specific exons during transcript processing, to by-pass DMD-causing dystrophin gene lesions and generate shorter, partially functional BMD-like dystrophin isoforms, and is showing promise as a therapy for DMD. Dystrophin gene structure in mildly affected and asymptomatic BMD patients indicates templates for a number of functional dystrophins, however, in-frame deletions in some regions of the dystrophin gene, particularly 5' of exon 55 are rare and the consequences of exon exclusion in this region are not known.

The *mdx* mouse is a widely used dystrophinopathy model and has a nonsense mutation in dystrophin exon 23. AO induced-excision of this exon from the mRNA removes the mutation without disrupting the reading frame, resulting in functional dystrophin expression and amelioration of the phenotype. We now report that systemic administration of AO combinations to wild-type mice can remove dystrophin exons to generate DMD- and BMD-like dystrophin isoforms for functional evaluation. Assessment of contractile properties of the muscle reveals that some in-frame exon combinations confer near normal function, while others result in muscle susceptible to contraction-induced damage. Furthermore, we show that concurrent administration of prednisolone and AOs improves dystrophin exon skipping and muscle function.

#### **Muscle Satellite Cells**

#### **Poster 20**

##### **The host muscle environment has got a profound effect on satellite cell function**

L. Boldrin<sup>1</sup>, P. S. Zammit<sup>2</sup>, F. Muntoni<sup>1</sup>, J. E. Morgan<sup>1</sup> <sup>1</sup>*Dubowitz Neuromuscular Centre, Institute of Child Health, University College London, London, UK* <sup>2</sup>*Randall Division of Cell and Molecular Biophysics, King's College London, London, UK*

Satellite cells are able to regenerate skeletal muscle and repopulate the satellite cell compartment, but their capacity to do this is profoundly influenced by the local host muscle environment. We have used the dystrophin-deficient *mdx* mouse, a model of Duchenne muscular dystrophy, to test the contribution of normal donor satellite cells to regenerated muscle fibres. The muscles of *mdx* mice undergo cycles of degeneration and regeneration, commencing at approximately three weeks of age. However, donor cells neither efficiently regenerate myofibres, nor self-renew, when grafted into host *mdx nu/nu* muscles.

In an attempt to improve the engraftment efficiency of donor satellite cells, we have modified the host muscle environment in ways that model the changes that occur in dystrophic muscles. These included: high dose of radiation, which incapacitates the majority of resident satellite cells and prevents endogenous muscle regeneration; cryoinjury, which damages locally both muscle fibres and satellite cells but allows muscle regeneration; injection of myotoxins, which cause fibre necrosis, but permit host muscle regeneration.

Donor satellite cells grafted into irradiated *mdx nu/nu* host muscles both efficiently regenerated skeletal muscle and self-renewed. However, significantly less donor-derived muscle regeneration occurred in host muscles that had been either cryodamaged or injected with myotoxins. Activation of radiation-resistant host satellite cells did not alter the number of donor-derived myofibres compared to irradiation. These data imply that an empty host satellite cell niche is not an essential requirement for efficient donor satellite cell function.

## Poster 21

### **ErbB3 binding protein-1 (Ebp1) contributes to the control of proliferation and differentiation in adult muscle satellite cells**

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Satellite cells (SC), the resident skeletal muscle stem cell, play key roles in postnatal skeletal muscle growth, hypertrophy and repair. In adult, SC are in close association with skeletal muscles fibers and are normally mitotically quiescent. In response to different stimuli (e.g. injury), SC become activated, proliferate extensively and then fuse with each other to form new myofibers or fuse to damaged myofibers to repair them. Some SC escape immediate differentiation and instead return to a quiescent state, in order to maintain a reserve of SC for future need (self-renew). Owing to their remarkable capacity to regenerate muscle, satellite cells have been considered as powerful candidates for cell-based therapies to treat muscular diseases. However, the efficient use of satellite cells requires a better understanding of the molecular mechanisms that control their proliferation, differentiation and self-renewal.

The ErbB3 receptor and its ligand Neuregulin, are involved in muscle growth (protein synthesis) and are important for myoblast differentiation. Ebp1 (ErbB3 binding protein-1) is a DNA/RNA binding protein implicated in cell growth, apoptosis and differentiation. Ebp1 has 2 isoforms, p48 and p42, that exhibit different cellular activities. Here we have investigated the role of Ebp1 in regulating SC function. The larger p48 isoform only, is expressed in SC and C2C12 myoblasts, being located in both the cytoplasm and the nucleus. While not present in quiescent SC, P48 is strongly induced during activation, remaining at high levels during proliferation and differentiation. Ebp1 Knockdown by siRNA inhibited both proliferation and differentiation of SC and C2C12 myoblasts, with a clear failure in myotube formation observed. While ErbB3 receptor levels were significantly reduced, over-expression of ErbB3 in Ebp1 knockdown cells was unable to rescue the differentiation defect. These results indicate that Ebp1 plays an important role in SC proliferation and differentiation, but this function is largely independent of ErbB3 receptor signaling.

## Poster 22

### **Age and Sex Related Differences in Satellite Cell Number, Proliferation and Self Renewal**

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Satellite Cells (SCs) are defined by their anatomical location between the sarcolemma and the basal lamina of muscle fibres. Evidence suggests that only a subpopulation of SCs function as muscle stem cells. Here we demonstrate that the number of SCs per muscle fibre declines with age and is decreased in females compared to males. Single fibre suspension cultures show no difference in the kinetics of SC activation or the amount of self renewal. Together, these data suggest that changes in SC number do not reflect changes in SC function. Moreover, engraftments into pre irradiated muscles of mdx nude mice show no difference in the regenerative capacity of SCs obtained from male or female, pre weaned or adult donors. Despite variance in the total number of SCs, the number of 'stem' satellite cells remains constant. Current work aims to correlate SC marker expression with changes in the ratio of 'stem' to 'non stem' SCs to uncover possible stem SC isolation criteria.

## **Muscle Channelopathies and Myasthenia Gravis**

## Poster 23

### **Fluorescent receptors to light up the neuromuscular junction**

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The neuromuscular junction (NMJ) is a highly specialised structure allowing rapid and efficient transmission of signal from the nerve to the muscle. Mutations in NMJ proteins may cause congenital myasthenic syndromes (CMS). In slow channel CMS, mutations in the AChR subunits alter the channel kinetics by prolonging the burst duration, thereby causing cationic overload and leading to endplate myopathy.

In order to test new therapies and analyse the mechanisms of existing therapies for slow channel syndrome, we generated a transgenic mouse expressing the slow channel mutation L221F in the human  $\alpha$ -subunit of the AChR. EGFP was inserted into the large cytoplasmic loop, located between the third and fourth transmembrane domains, to allow visualisation of the mutant  $\alpha$  subunit ( $\alpha$ L221F-EGFP). Transgene transcription was driven from the AChR  $\alpha$ -subunit promoter to restrict expression to the post-synaptic nuclei in skeletal muscle. Transgenic mice were back-crossed with mice deficient in endogenous AChR, to generate mice that expressed only mutated  $\alpha$ -subunit. The mice have no obvious defects at birth, thrive and survive for longer than 12 months but show fatiguability. Analysis of endplates from EDL, soleus and diaphragm muscles using fluorescently tagged  $\alpha$ -bungarotoxin showed that  $\alpha$ L221F-EGFP is correctly incorporated into endplate AChR. Electron microscopy showed a large variability in endplate morphology within the same muscle. Electrophysiological analysis demonstrated prolonged decay of the EPPs and MEPPs, thereby confirming slow channel characteristics of the  $\alpha$ L221F-EGFP receptors. Our model accurately reflects the disease in humans and is currently being used to test the efficacy and mechanisms of novel and existing slow channel therapies.

## Poster 24

### **Genetic heterogeneity and mechanisms of phenotypic variability in human skeletal muscle channelopathies**

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Background: Skeletal muscle channelopathies are a group of neuromuscular disorders that are caused by mutations of voltage gated ion channels resulting in altered membrane excitability. They include the non dystrophic myotonias and the periodic paralyses. There are two main types of

periodic paralysis: hyperkalemic periodic paralysis (HyperPP) and hypokalemic periodic paralysis (HypoPP) which are the result of mutations in the genes coding for the skeletal muscle voltage gated calcium channel (CACNA1S) or sodium channel (SCN4A). The causative mutations of HypoPP have been found to localise in the voltage sensing segments (S4) of SCN4A and CACNA1S. However, the causative mutations for other skeletal muscle channelopathies have not been found to cluster in such a localized area of the causative gene.

**Aims:** To investigate genetic heterogeneity in the skeletal muscle channelopathies and potential mechanisms for phenotypic variability including differential allelic expression and abnormal protein trafficking.

**Methods:** Exonic sequencing of causative genes will be used to find additional mutations within these genes that cause skeletal muscle channelopathies. Phenotypic variation will be looked at using qPCR and immunohistochemistry of muscle samples.

**Initial Results:** SCN4A and CACNA1S S4 segments in an initial group of patients were sequenced and two novel mutations of SCN4A were found; p.S653G in a patient with HyperPP and p.R222Q in a patient with Myotonia Congenita. This is only the second SCN4A S4 arginine mutation to be reported in a phenotype other than HypoPP. R222W has previously been described, in HypoPP (Matthews et al 2009). Functional studies of the variability in voltage sensor function and resultant phenotype with differing residues may lead to better understanding of the pathomechanisms of disease.

## Poster 25

### **Double-blind, placebo-controlled, parallel group, phase III study comparing dichlorphenamide vs. placebo for the treatment of periodic paralysis (HYP HOP trial)**

J. Burge<sup>1</sup>, A. Horga<sup>1</sup>, RC Griggs<sup>2</sup>, M.G. Hanna<sup>1</sup>. *On behalf of the Hyp Hop Investigators*. <sup>1</sup>MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK and <sup>2</sup>University of Rochester NY USA.

The periodic paralyses (PP) are inherited muscle channelopathies that usually present in the first two decades of life with episodes of focal or generalized weakness. A progressive myopathy may develop later in the disease course. Preliminary studies on a limited number of participants suggest that carbonic anhydrase inhibitors, such as acetazolamide and dichlorphenamide (DCP), are effective in the prevention of episodic weakness in PP.

The purpose of this multicentre Phase III clinical trial is to assess the efficacy, safety and tolerability of DCP vs. placebo in patients with both hyperkalemic and hypokalemic PP.

Participants will be randomized to receive oral DCP or placebo during 9 weeks. The primary outcome measure for this period is the rate of episodes of weakness as measured by participant self-report over the last 8 weeks. This 9-week phase will be followed by a 1-year open-label extension phase without placebo to determine the long-term effects of DCP on the disease course and interictal weakness.

We aim to recruit a total of 40 patients in the UK as part of 140 patients recruited internationally at 12 centres in the US and Europe. As of January 2010, 25 patients have been enrolled across all study sites, and 15 of them are in the open-label extension phase. We herein present a progress report on the HYP HOP trial at the UK site.

## Poster 26

### **Pyridostigmine-responsive limb-girdle congenital myasthenic syndrome with frequent tubular aggregates**

Juliane Müller<sup>1</sup>, Jan Senderek<sup>2,3</sup>, Marina Dusl<sup>4</sup>, Velina Guergueltcheva<sup>4,5</sup>, Steve Laval<sup>1</sup>, Kate Bushby<sup>1</sup>, Volker Straub<sup>1</sup>, David Beeson<sup>6</sup>, Angela Abicht<sup>4</sup>, Hanns Lochmüller<sup>1</sup> <sup>1</sup>Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK <sup>2</sup>Institute of Human Genetics, RWTH Aachen University, Aachen, Germany <sup>3</sup>Institute of Cell Biology, ETH Zürich, Switzerland <sup>4</sup>Friedrich-Baur-Institut, Department of Neurology, Ludwig Maximilians University, Munich, Germany <sup>5</sup>Clinic of Neurology, University Hospital Alexandrovska, Sofia, Bulgaria <sup>6</sup>Neurosciences Group, Weatherall Institute of Molecular Medicine, Department of Clinical Neurology, University of Oxford, UK



Congenital myasthenic syndrome (CMS) is a clinically and genetically heterogeneous group of inherited muscle disorders caused by genetic defects at the neuromuscular junction. To date mutations in twelve different genes were identified as responsible for CMS. We have established a large CMS patient cohort of more than 850 patients from all over the world; approximately 50% of these patients have been genetically diagnosed so far.

One as yet genetically undiagnosed CMS subgroup is pyridostigmine-responsive limb-girdle congenital myasthenia (LG-CMS). We have characterised 21 patients from 13 families in our cohort with this clinical phenotype. All families show an autosomal recessive disease inheritance pattern. All patients have proximal limb-girdle weakness and no ophthalmoparesis or bulbar weakness. In the majority of the patients muscle biopsies disclose tubular aggregates arising from the sarcoplasmic reticulum. The patients respond well to cholinesterase inhibitor treatment. Direct sequencing or haplotype analysis excluded all known genes and loci involved in CMS to date. A genome-wide linkage screen in one extended pedigree mapped the disease locus to a 15.75 Mb interval on chromosome 2p12-p15. All of the smaller families were compatible with linkage to this locus and allowed to refine the new LG-CMS locus to a suggestive critical region of 5.92 Mb. Our study confirms that LG-CMS with tubular aggregates is a distinct clinical and genetic entity. The identification of the underlying gene mutations responsible for pyridostigmine-responsive LG-CMS with TAs will further enhance our understanding of this disorder.

#### **Poster 27**

##### **Genotype-phenotype correlation and longitudinal three year natural history study in the Non-dystrophic myotonias in the UK**

D.L. Raja Rayan, E. Matthews, S. Rajakulendran, G. Barreto, S.V. Tan, L. Dewar, J. Burge, R.C. Griggs, R. Barohn, M.G. Hanna and the CINCH group, *MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*

The Non-dystrophic Myotonias (NDM) are a rare group of genetic muscle diseases caused by mutations in the sodium or chloride ion channel genes, SCN4A and CLCN1. They are characterised clinically by stiffness due to impaired muscle relaxation and are often accompanied by pain and weakness. There is little information available regarding the natural history of the disease making a study of this magnitude timely. The heterogeneous nature of these diseases makes diagnosis difficult and therefore assessing genotype-phenotype correlation is an important aspect in understanding NDM.

This multi-centred National Institute of Health supported study is part of an international collaboration as members of the Clinical Investigation of Neurological Channelopathies (CINCH) group. It aims to characterise, with a standardised protocol, the clinical and electrical features of NDM; establish the natural history of these diseases and identify key genotype-phenotype correlations over a 3 year period which is now completed. In the UK we have recruited 20 patients with genetically-confirmed NDM, and across the 6 sites in the US and UK, 95 patients have been recruited and followed for 3 years. In our cohort, we have 7 with myotonia congenita, 7 with paramyotonia congenita, 4 with sodium channel myotonia and 2 with myotonic dystrophy type 2. Our initial analysis of these patients' data show distinct clinical features and electrophysiology patterns for each sub-group of patients helping to establish genotype-phenotype correlations. Further analysis will help us identify key endpoints for future trials and improve diagnosis and management of NDM.

#### **Poster 28**

##### **Assessing the efficacy of Mexiletine in UK patients with Non-dystrophic Myotonia**

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Non-dystrophic myotonia (NDM) is the commonest group of muscle channelopathies. Patients develop muscle stiffness due to delayed relaxation of the muscle following voluntary contraction which can significantly impair function. Mexiletine is a use-dependent sodium channel blocker with uncontrolled clinical trial evidence suggesting it may improve symptoms in NDM. At present there is

no high quality data on treatment in NDM. We evaluated the efficacy of mexiletine in our NDM patients in a double-blinded placebo controlled cross-over study in collaboration with the CINCH (Clinical Investigation of Neurological Channelopathies) study group.

We have recruited 14 patients in the UK as part of 60 patients recruited internationally at 7 centres. All patients were randomised to a 4 week course of mexiletine or placebo with a one week wash out period followed by a 4 week course of the drug they did not previously receive. The primary outcome measure was patient reported stiffness. Secondary outcome measures included patient reported weakness, tiredness and pain; quality of life measures; a clinical myotonia assessment; quantitative grip assessment and neurophysiology measuring long and short exercise tests and needle EMG pre and post each treatment leg.

The initial findings of the study demonstrates that mexiletine is safe in patients, with no serious adverse events reported and only mild side effects including nausea, tremor and exacerbation of migraines. There were no significant cardiological events. Once completed, the study aims to evaluate the efficacy of mexiletine in reducing stiffness in non-dystrophic myotonia.

### **Poster 29**

#### **Genotype-phenotype correlation and longitudinal study of Andersen-Tawil Syndrome in the UK**

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Andersen-Tawil Syndrome (ATS) is a rare ion channel disorder with 60% of cases commonly associated with mutations in the KCNJ2 gene affecting the Kir2.1 potassium channel. It is characterised by the triad of periodic paralysis, cardiac arrhythmias and skeletal developmental abnormalities. Episodes of paralysis can cause significant impairment of function and cardiac arrhythmias can be life-threatening but little effective treatment exists. At present there are no comprehensive longitudinal studies in ATS. As members of the Clinical Investigation of Neurological Channelopathies (CINCH) group we are involved in a large-scale multicentre National Institute of Health supported study in which we aim to identify the natural history of the disease and record genotype-phenotype correlation.

At present we have recruited 11 patients in the UK and overall 23 patients have been recruited over 6 centres in the US and UK. Patients across all sites are analysed using a standardised protocol to prospectively assess clinical symptoms and signs, quantitative muscle strength, neurophysiology and cardiological status over 3 years. Initial analysis demonstrates that all patients have skeletal abnormalities and episodes of weakness and 89% of these also had a positive McManis test. Cardiac symptoms were less common with 55% of patients having symptoms or ECG changes but this did not correlate with genotype.

Initial data from this study gives important insights into the genotype-phenotype correlations and prevalence of symptoms in ATS patients. Further data will provide vital information into the natural history of this condition and help define effective endpoints for further trials.

### **Poster 30**

#### **Large scale chloride channel gene DNA rearrangements are an important cause of recessive Myotonia Congenita- implications for diagnostic screening**

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Myotonia congenita (MC) is the commonest of the muscle channelopathies. It is caused by mutations in the chloride channel gene, CLCN1, causing stiffness that characteristically improves with exercise and myotonia on EMG. It can be either autosomal dominantly or recessively inherited. An important

diagnostic dilemma in recessive MC is that approximately 14% of cases only have a single mutation identified, despite sequencing of all 23 coding exons CLCN1.

Possible explanations include the presence of copy number variation (not identified by Sanger sequencing), unidentified mutations (eg in promoter regions or deep within introns) or possibly differential allelic expression. We have initially investigated if copy number variation (large scale rearrangements) may account for these cases using multiplex ligation dependent probe amplification (MLPA).

We have identified the first reported group of patients with large scale rearrangements in MC. In our series of 28 recessive cases that only harbour a single recessive mutation we identified copy number variation in 2. Overall we found that 6% of MC cases also carry large scale rearrangements. This includes 2 families with the homozygous mutation Pro744Thr and a triplication of exons 8 to 14 with onset of MC in the first years of life and severe myotonia.

We conclude that large scale deletions and duplications may explain some cases of recessive MC that were previously only considered to harbour a single recessive mutation. MLPA analysis is therefore an important diagnostic test for elucidating the cause of recessive myotonia congenita and should be included in genetic screening in recessive myotonia congenita.

### Poster 31

#### **Synaptic mechanisms in P/Q deficient neuromuscular junctions**

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Lambert Eaton Syndrome (LEMS) is an a neuromuscular disorder caused by antibodies that target voltage gated Ca<sup>2+</sup> channels at motor nerve terminals. P/Q type calcium channels are primarily involved in ACh release at the adult mammalian neuromuscular junction and are the main target of antibodies in LEMS. A variable proportion of LEMS patients have antibodies to N type channels. The CACNA1A gene encodes the pore forming  $\alpha$ 1A subunit of P/Q calcium channels. Elimination of this subunit in mice causes a rapidly progressive neurological syndrome characterised by dystonia, ataxia and weakness, resulting in death after 21 days

Our aim was to understand the biophysical consequences of antibody mediated disruption of presynaptic calcium channel function in LEMS.

The contributions of individual calcium channels to post synaptic currents in primary neuronal cultures were defined in CACNA1A knockout mice and their wildtype littermates. Excitatory post synaptic currents (EPSCs) were measured after sequential application of toxins to block P/Q, N, R and L type channels.

There was a significant increase in the contribution of N type calcium channels to synaptic release in CACNA1A knockout cultures compared to WT controls.

Having established this model, the next step is to incubate CACNA1A knockout and WT cultures with LEMS IgG to examine the effect of LEMS antibodies on synaptic transmission in P/Q deficient and WT neurons.

### Poster 32

#### **Myasthenic crisis in the intensive care unit - a ten year review**

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Introduction: The prognosis of autoimmune myasthenia gravis (MG) has improved with a reduction in mortality from 75% to a current rate of less than 5%. However, approximately 20% of MG patients will experience a myasthenic crisis requiring intensive care admission during the course of their illness.

Aim: To perform a 10 year audit on cases of myasthenic crisis admitted to a neurological intensive care unit.

Methods: Cases of myasthenic crisis were identified from the ICU register and case notes were retrospectively reviewed

Results: Thirty five patients were admitted in myasthenic crisis. Three patients had multiple admissions. The average age at admission was 56.7 years. Twenty three were female. Twelve were male. Average disease duration at ICU admission was 2.9 years. 80% of patients were acetylcholine receptor antibody positive. 11% tested positive for MuSK antibodies and 9% were seronegative. 30% of patients had a history of thymectomy. All patients were admitted in respiratory failure. 43% of patients had a precipitating systemic infection. One patient had an associated myocardial infarction. 60% required invasive mechanical ventilation. 86% received IVIG and 9% were treated with plasma exchange. The average duration of ICU stay was 13.8 days. There were 3 deaths. Conclusion: MG continues to be a serious disease with a considerable morbidity and mortality. Management of myasthenic crisis requires specialised neuro-intensive care facilities.

### Poster 33

#### **Impaired neurotransmission in a mouse model of the slow channel congenital myasthenic syndrome is improved by the sympathomimetic drug ephedrine**

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The sympathomimetic drug ephedrine has been reported as beneficial in congenital myasthenic syndromes (CMS) due to mutations in *DOK7* and in *COLQ*. These CMS both may worsen in response to treatment with cholinesterase inhibitors. The slow channel CMS (SCCMS) a progressive form of CMS, in which acetylcholine receptor channel openings are prolonged, also worsens after cholinesterase inhibitors. We sought to test the hypothesis that ephedrine would improve neurotransmission in our mouse model of SCCMS (see accompanying poster by Cossins *et. al.*). SCCMS mice at 6 weeks of age were compared to wild-type mice for inverted screen endurance (measuring fatigable muscle weakness) and two electromyographic (EMG) measures of neurotransmission (compound muscle action potential (CMAP) decrement and single fibre jitter). SCCMS mice received 6 weeks oral ephedrine treatment (25mg/Kg/day, bioavailability differs between humans and mice) and were compared to untreated SCCMS mice. EMG studies were performed before and after treatment.

For all three parameters SCCMS mice were significantly impaired vs wild-type. Inverted screen duration was  $241 \pm 23$  s (n=35) whilst all wild-type mice achieved the maximum 600 s (n=7), CMAP decrement at 10 Hz stimulation frequency was  $40.9 \pm 3.4$  % vs.  $1.6 \pm 2.4$  % and single fibre jitter was  $16.1 \pm 0.6$  □s vs.  $8.6 \pm 0.5$  □s.

Following ephedrine treatment inverted screen duration and single fibre jitter improved significantly ( $p < 0.05$ , n=10, paired t-test) ( $198 \pm 37$  s to  $289 \pm 61$  s screen duration &  $16.4 \pm 1.1$  □s to  $14.1 \pm 1.0$  □s for jitter) whilst untreated mice showed no significant change. In ephedrine treated SCCMS mice CMAP decrement was unchanged whilst in untreated mice CMAP decrement significantly deteriorated ( $33.6 \pm 6.1$  to  $51.7 \pm 5.6$  %, n=11). These data suggest that ephedrine should improve neurotransmission in SCCMS patients.

## **Peripheral Nerve Disease**

### Poster 34

#### **Clinical phenotype and novel mutations in *Alsin* related motorneuron disease**

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neuron involvement (JPLS; juvenile primary lateral sclerosis or JALS; juvenile amyotrophic lateral sclerosis).

We have recruited a cohort of 11 patients from 7 Turkish families with mainly IAHS phenotype and have performed genomic sequencing of *ALS2* after linkage to its locus.

Our study unveiled five novel and one known pathogenic mutations in the *ALS2* gene in six families (6/7). The age range at clinical assessment was 5-20 years. The disease began mostly with delayed walking around 2 years of age accompanied by tiptoe walking. Interestingly, one patient was reported to be floppy shortly after birth and another one never achieved independent walking. All patients had developed upper limb involvement during disease course, although weakness and spasticity were more prominent in the lower limbs with clear associated pyramidal signs. Severe dysarthria or even anarthria was a common feature in the first decade of life, accompanied by facial weakness and pronounced dysphagia. Cerebral MRI and EEG were normal in all but one patient, who had epilepsy.

*Alsin* functions as guanine nucleotide exchange factor and it is involved in endosomal dynamics. So far fourteen mutations have been reported in *ALS2*, being mostly small indels. Here, we report a splice site mutation (IVS5+1G>A), three missense mutations (p.Cys157Tyr, p. Ala573Glu and p.Leu1054Pro), one nonsense mutation (p.Tyr348X) and one insertion (c.4574insG), all in homozygous state. There was no obvious genotype-phenotype correlation. Our study indicates a quite high detection rate of *Alsin* mutations in clinically well characterized infantile onset motor neuron disease. Genetic investigations could provide accurate diagnosis for patients and families; additionally contribute to better understanding of the pathophysiology of HSP, PLS and ALS.

### Poster 35

#### **Microfluidic chambers provide a novel method to study the functional properties of sensory neuron terminals in culture**

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The transduction properties of sensory cells are commonly studied in vitro using dissociated DRG cultures. In this system, the soma is used as a model of peripheral action potential transduction, however molecular and physiological observations made in the cell body may not relate to those in the sensory nerve terminal. Strikingly, little is known about the similarities and differences of transduction properties of the soma and neuronal terminals of sensory neurons. Therefore, we have set out to develop a system that allows functional investigations of neuronal properties in segregated compartments containing either cell bodies or neurites.

We use microfluidic culture platforms which provide an ideal way to study the axons of sensory neurons in isolation to the soma. Fluidically isolated compartments are linked by microgrooves of 3 µm height, 10 µm width and 800 µm length. The dissociated DRG cultures are seeded out into the somal compartment and allowed to adhere. A neurotrophin gradient across the microgrooves encourages axons to grow through the microgrooves into an adjacent compartment. Once the axons are established, they can be treated and investigated separately to the cell body.

Action potential propagation in the compartmentalised system relies on voltage gated sodium channels, with 20% of neurons displaying TTX-resistant characteristics. Furthermore, the study of sensory neuron terminals using functional calcium imaging has revealed a significant differential regulation of capsaicin sensitivity on the terminals compared to the cell soma. A population of sensory terminals also rely on TRPV1 independent mechanisms for proton transduction.

We conclude that microfluidic chambers are a reliable model system that will allow examination of functional properties of the terminals of sensory neurons in vitro.

### Poster 36

#### **TRPV4 Mutations and Functional Characterisation in a Cohort of Patients with Hereditary Neuropathy**

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TRPV4 mutations have recently been identified in a range of inherited peripheral neuropathies with additional clinical features. However, the underlying pathological mechanism remains unclear. We aimed to identify and characterise TRPV4 mutations by sequencing TRPV4 coding regions and splice junctions in 269 patients with Charcot-Marie-Tooth disease type 2 (CMT2), 95 patients with distal hereditary motor neuropathy (dHMN) and 352 controls. Seven patients were heterozygous for putative pathogenic TRPV4 mutations absent from controls. Of these variants, six were novel and one (R232C) had been found previously in two families with HMN and Scapuloperoneal Spinal Muscular Atrophy (SPSMA). Two of the variants (R232C and Y567X) segregated with disease in patient families. R232C was detected in a female with CMT2 and is located within the ankyrin repeat domain (ARD), thought to be involved in oligomerisation, protein interactions, and trafficking of TRPV4 to the plasma membrane. Y567X was present in a male and his father, both with CMT2. The Y567X was absent from their cDNA suggesting nonsense-mediated decay of the mutant transcript. It is therefore possible that haploinsufficiency of TRPV4 can contribute to the development of neuropathy. One variant, E218K, did not segregate with disease in the patient's family demonstrating that not all rare TRPV4 non-synonymous variants are associated with disease. To our knowledge this is the largest study to date screening patients with CMT2 and dHMN for TRPV4 mutations. Our results demonstrate that TRPV4 variants have a wide clinical spectrum and are present in approximately 2% of patients with CMT2 or dHMN.

### Poster 37

#### **Phenotype in E410K Beta-tubulin isotype 3 mutations: striking facial weakness and other extraocular manifestations in addition to CFEOM**

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Introduction: The Congenital Cranial Dysinnervation Disorders (CCDDs) are congenital, non-progressive disorders resulting from developmental abnormalities of one or more cranial nerves/nuclei with primary or secondary dysinnervation and include Duane's syndrome, Möbius syndrome, Congenital Fibrosis of the External Ocular Muscles (CFEOM) and Congenital ptosis. Recently mutations in the Beta-tubulin isotype 3 (TUBB3) gene have been reported to produce several phenotypes depending on the particular mutation.

Methods: Sequence analysis of the TUBB3 gene was undertaken in CCDD families with CFEOM phenotypes for which a genetic cause had not previously been determined.

Results: Four affected individuals in two families were identified to have a previously reported heterozygous missense TUBB3 mutation, E410K. The phenotype in all individuals consisted of CFEOM with some variability but preserved eye abduction bilaterally and striking facial weakness. Other findings included developmental delay, corpus callosum hypoplasia and an adult with sensory axonal neuropathy.

Conclusion: There are dramatic genotype/phenotype correlations depending on the particular TUBB3 mutation. Although there was striking facial weakness in our cases with ocular involvement they did not fulfil criteria for Möbius syndrome as abduction was preserved. In cases where CFEOM is part of the phenotype, TUBB3 mutations increase microtubule stability.

### Poster 38

#### **Charcot-Marie-Tooth Disease and Related Disorders: A Natural History Study**

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Charcot-Marie-Tooth disease (CMT), distal hereditary motor neuropathy (dHMN) and hereditary sensory and autonomic neuropathy (HSAN) are a clinically and genetically heterogeneous group of

disorders. At present, mutations in more than 40 causative genes have been identified. Despite these striking advances, there are no effective treatments for any type of CMT, dHMN and HSAN. Natural history data are available for only the most common types (CMT1A and CMTX) and many genotype-phenotype correlations remain unknown. To address these issues a Rare Disease Clinical Research Network (RDCRN) has been established. The RDCRN includes expert researchers in CMT belonging to centres in US, UK, Australia and Italy. The aims of the projects are: 1) to characterize the natural history of various forms of CMT, dHMN and HSAN; 2) to establish genotype-phenotype correlations; 3) To develop a paediatric neuropathy score (CMTPedS); 4) to identify new genes and genetic modifiers in CMT; 5) to train neurologists in inherited neuropathies. Patients will be yearly assessed over 5 years. Evaluations consist of neurological history and examination, nerve conduction studies, completion of minimal dataset, CMT Neuropathy Score (CMTNS), CMTPedS, quality of life questionnaires. Blood will be taken from selected patients to look for new genes and for genetic modifiers. In selected cases a skin biopsy will be performed to investigate the pathogenic mechanism of a particular mutation. Accrual has been successful so far with 400 patients recruited and 105 children assessed by the CMTPedS. DNA samples from 213 patients will be analysed for genetic modifiers and novel genes.

### Poster 39

#### **Neurofilament light chain polypeptide gene (*NEFL*) mutations in autosomal dominant or sporadic Charcot-Marie-Tooth disease**

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**Background** The neurofilament light chain polypeptide gene (*NEFL*), encoding the major intermediate filament in neurons and axons, is one of the causative genes of Charcot-Marie-Tooth disease (CMT) and the main gene associated with CMT2E/1F. *NEFL* mutations are usually inherited in an autosomal dominant manner; however, de novo mutations can occur. In most affected individuals, the typical phenotype includes early onset within the first decade of life, severe sensorimotor dysfunction and severely to moderately reduced nerve conduction velocities. . Nevertheless, recessive inheritance has been reported, too.

**Objective** The aim of this study was to screen *NEFL* in a cohort of CMT patients without a genetic diagnosis, including demyelinating, axonal, and intermediate subtypes.

**Methods** We investigated *NEFL* in a cohort of 184 CMT patients with autosomal dominant or sporadic CMT. The cohort included patients with CMT1, CMT2 and intermediate CMT. All were negative for mutations in *PMP22*, *MPZ*, *GJB1*, *MFN2*, and the *SH3TC2* hotspot.

**Results** We detected one novel heterozygous missense mutation, Leu83His, in a patient with sporadic CMT1.

**Conclusion** This study confirmed that *NEFL* mutations are a rare cause of CMT; however, screening should be considered in patients with CMT, particularly if the patient has early onset and is negative for the other known CMT genes even when there is no contributory family history.

### Poster 40

#### **A family with a TRPV4 related neuropathy displays marked phenotypic variability ranging from profound neuromuscular disability to non-penetrance**

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**Background:** Recently mutations in TRPV4 has been identified as causing two distinct groups of disorders; namely TRPV4 related neuropathy spectrum (encompassing HMSN IIC, SPSMA and congenital distal SMA) and a family of autosomal dominant short stature skeletal dysplasias that show prominent vertebral involvement. Vocal cord paralysis is a key feature in the individuals

affected by the neurological disorder. Scoliosis, arthrogyrosis and talipes have also been observed in this latter group leading to the proposal that these changes might have a skeletal rather than a neurological cause.

Objective: To compare and contrast the clinical and molecular findings in a new family with a TRPV4 mutation with the published literature on this rare group of disorders.

Methods: 5 members of the family available for study underwent clinical and molecular evaluation. Electrophysiology studies were carried out in 2 affected individuals and expert radiologist opinion was obtained on X rays from 3 affected individuals.

Results: The R315W mutation in TRPV4 was identified in all 5 individuals studied. The phenotype in mutation carriers varied from severe neuromuscular disability to non-penetrance. Vocal cord paralysis was present in 4/5 and skeletal deformity affecting the chest and or spine in 3/5 individuals studied. Scrutiny of the x-rays did not show evidence of abnormal bone modelling.

Discussion: The findings in this family confirm previous observations of marked intrafamilial clinical variability in TRPV4 related neuropathy and provide further evidence that non-penetrance is a frequent observation in this condition. Examination of this family did not find evidence for an overlap with the skeletal dysplasia group.

## Poster 41

### Genetic mutation frequency in patients with Hereditary Sensory and Autonomic Neuropathies (HSAN)

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Hereditary sensory and autonomic neuropathies (HSAN, also known as hereditary sensory neuropathies, HSN) are a clinically and genetically heterogeneous group of disorders. To date, mutations in nine genes have been identified as being responsible for causing hereditary sensory neuropathies. We selected a cohort of 149 patients diagnosed with hereditary sensory neuropathies. We screened these patients for mutations in the coding regions of the known HSN genes, *RAB7*, *WNK1/HSN2*, *NTRK1*, *NGFB*, *FAM134B* and *SPTLC1*. We found mutations in 24 (16%) index cases, a similar frequency to that reported by Rothier et al (19%). Mutations in *SPTLC1* were the most frequent cause of HSN in this cohort with a frequency of 12%, followed by *NTRK1* with a frequency of 6%. Mutations in other genes (*FAM134B*, *WNK1/HSN2*, *NGFB*, *RAB7*) caused <2% each. This illustrates that mutations in most of the known HSN genes are rare and that more HSN genes remain unknown.

## Poster 42

### X-inactivation pattern in females with CMTX1

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X-linked Charcot-Marie-Tooth disease due to mutations in the *GJB1* gene (CMTX1), is the second most frequent cause of CMT. Males have a uniform phenotype with moderate severity which progresses with age. Females, however, often have a less severe neuropathy and some are asymptomatic, although others are as severely affected as males. This variable phenotype in females has been suggested to occur as a result of X-inactivation. X-inactivation occurs randomly during development and results in either the paternally- or maternally- inherited X chromosome being inactivated in each cell. Approximately 20% of unaffected women have skewed X-inactivation such that the ratio of X-inactivation is  $\geq 80:20$ . Carrying a mutation in some X-linked genes is known to affect the X-inactivation pattern.



This study aimed to address whether carrying a *GJB1* mutation affects X-inactivation and whether there is a correlation between X-inactivation pattern and phenotype.

In order to determine whether carrying a *GJB1* mutation affects X-inactivation we compared X-inactivation patterns between females with CMTX1 and controls. We then compared the clinical phenotype as measured by the CMT examination score (CMTES, a subset of the CMT neuropathy score) with the percentage of mutant allele that was active in females with CMTX1 to see if there was a correlation between X-inactivation pattern and phenotype.

We examined X-inactivation pattern in 37 females with CMTX1 and 23 female controls. There was no significant difference in X-inactivation pattern between the two groups. In addition, there was no correlation between the proportion of mutant allele active and CMTES.

In conclusion, carrying a *GJB1* mutation does not affect X-inactivation pattern and X-inactivation pattern in blood does not appear to correlate with clinical phenotype as measured by the CMTES.

#### **Poster 43**

##### **Genetic modifying factors for the common form of CMT1A due to the chromosome 17 duplication and other causes of CMT1 in non-CMT1A patients**

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Background: Charcot-Marie-Tooth disease (CMT), the most common inherited neuromuscular disorder, is classified as either demyelinating (CMT1) or axonal (CMT2). CMT patients exhibit wasting of distal muscles, reduced reflexes, foot deformities, loss of sensation and walking difficulties. CMT1A varies widely phenotypically both within and between families, and is due to a 1.4 Mb duplication on chromosome 17 which includes the PMP22 gene. The demyelinating form of CMT may also be caused by mutations in *EGR2*, a transcription factor necessary for the development of Schwann cells.

Aims: The genetic modifying factors of the CMT1A phenotype are to be studied by analyzing a cohort of 1000 CMT1A cases. Copy number variants and their contribution to the CMT1A phenotype are being analyzed. Another aim is to assess whether the mRNA expression of the PMP22 gene is modulated by the duplicated CMT1A haplotype or other genes and is associated with the CMT1A phenotype.

To explore other causes of CMT1 in non-CMT1A patients, a cohort of patients is being screened for mutations in *EGR2*.

Methods: PMP22 and *EGR2* are being sequenced. Chip arrays are being used to study the association of 384 SNPs with disease phenotypes. High-density whole-genome SNP genotyping, copy number variant analysis and MLPA will be performed. PMP22 gene function and expression will be explored using fibroblast cultures.

Expected outcomes: Most variance is expected to be found within and nearby to the PMP22 gene. This project will help elucidate the pathogenesis of CMT1A by identifying the genetic factors that modulate the disease phenotype.

#### **Poster 44**

##### **Frequency and circumstances of falls for adults with Charcot-Marie-Tooth disease**

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Falls are anecdotally reported by people with Charcot-Marie-Tooth disease (CMT) but to date there has been little formal investigation of this problem. Frequent falling increases risk of injury and reduces mobility through avoidance of activities perceived threaten stability. To successfully manage the problem of falls there needs to be greater understanding of why people fall. This study aims to survey people with CMT to ascertain how often they fall, nearly fall and the circumstances that may have contributed.

A postal survey was administered to 222 people with CMT under the care of the MRC Centre for Neuromuscular Diseases. Ninety four questionnaires were returned.

In total, 89.4% of respondents reported falls, most commonly occurring at least once a month (29.8%), 19% fell more frequently than once a month and 33% less than once a month. Near falls in the previous six months were reported by 89.4% of respondents. A proportion of participants nearly fell once a month (27.7%), 42.6% more frequently than once a month and 19.1% less than once a month.

The majority of falls happened indoors (47.8%). Most falls or near falls occurred when people were walking (52.2%; 63.4%) and participants reported the causes as being ankles/knees giving way, tripping and loss of balance. The majority of falls resulted in no or minor injuries (61.2%).

Falls and near falls are regular events for many people with CMT. Peoples' mobility around their own home would require further investigation by clinicians to reduce the risk of future falls.

#### **Poster 45**

##### **A clinical study of the hereditary neuropathies due to mutations in the small heat shock proteins**

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**Introduction:** In the last decade, mutations in three small heat shock proteins (Hsp) HSPB1, HSPB3 and HSPB8 have been identified as causes of motor predominant forms of Charcot-Marie-Tooth type 2 disease (CMT2). Genetic testing in CMT2 and distal hereditary motor neuropathy (dHMN) can be laborious and expensive due to the large number of causative mutations, each with a similar phenotype. Identifying a diagnostic phenotype in patients with CMT2 is therefore of benefit in ensuring a timely and accurate genetic diagnosis.

**Methods:** 11 patients with dHMN and CMT2 due to HSPB1 mutations and 1 patient with dHMN due to a HSPB8 mutation were examined and underwent nerve conduction studies and EMG. 150 patients with distal hereditary neuropathy were screened for mutations in HSPB3, a recently reported gene mutated in dHMN.

**Results:** HSPB1 and HSPB8 associated neuropathies cause a length dependent motor neuropathy. For both HSPB1 and HSPB8 mutations the pattern of weakness was characterised by disproportionate ankle plantar flexion weakness compared to ankle dorsiflexion. This pattern of weakness has not been observed in other forms of dHMN or CMT2. No mutations in HSPB3 were identified in our cohort of dHMN patients.

**Conclusion:** Mutations in HSPB1 and HSPB8 cause a unique pattern of weakness whereby ankle plantar flexion is affected before ankle dorsiflexion.

#### **Poster 46**

##### **Tremor in Charcot Marie Tooth disease**

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**Objective:** Investigate tremor associated with Charcot Marie Tooth disease (CMT) and determine the role of the cerebellum and its connections in the pathophysiology of the tremor.

**Background:** Tremor is recognised as an accompanying symptom of some patients with CMT but the pathophysiology is unclear. Tremor in association with neuropathy is generally seen in demyelinating sensori-motor neuropathies; in patients with CIDP we have previously reported an abnormality in the cerebellar dependent task of classical eye-blink conditioning (EBC) in patients with tremor compared to those without. Here we used tremor analysis, EBC and a motor learning paradigm to study patients with CMT as an initial exploration of the occurrence and pathophysiology of tremor.

Methods: 2 groups of 10 participants were studied: Patients with CMT1a and age/sex matched healthy volunteers. Assessment involved: (a) clinical assessment, (b) rating of neuropathy and tremor, (c) tremor recording using hand accelerometry and surface EMG, (d) EBC, (e) rotation learning paradigm using a joystick.

Results: Tremor was present, though mildly, in most (60%) CMT patients. Group-wise comparison of EBC revealed a low rate of conditioning in the CMT group as a whole compared to normal controls, but this abnormality did not differ between CMT patients with and without tremor ( $p=0.024$ ). Despite abnormal EBC, rotation learning, a task thought to depend in part on the cerebellum, was normal in CMT patients. The presence and severity of tremor was not associated with the severity of neuropathy or presence of pseudoathetosis. Inertial loading of a limb led to an increase in amplitude of tremor in both the loaded and un-loaded limbs in most CMT patients.

Conclusions: In contrast to previous data in CIDP, both patients with CMT with and without tremor have abnormal EBC. These results, combined with the unexpected increase in tremor amplitude with loading in CMT, suggests that there may be different mechanisms for generation of tremor in different types of neuropathy.

## **Mitochondrial Disease**

### **Poster 47**

#### **Mutations in the novel chaperone *FOXRED1* cause mitochondrial complex I deficiency**

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*Introduction:* Mitochondrial complex I deficiency is the most common defect of the oxidative phosphorylation system. Clinical presentation of complex I defects include a broad array of heterogeneous phenotypes. Currently causative mutations are identified in fewer than 50% of cases: ~25% have mutations in mitochondrial DNA, another ~20% have mutations in 12 of the 38 nuclear-encoded structural subunits of the enzyme, and rarely disease may be caused by defects in an assembly factor.

*Material and methods:* We describe a patient with complex I deficiency from a consanguineous Iranian-Jewish pedigree who presented with infantile-onset encephalomyopathy associated with lactic acidosis and 7% residual complex I activity in skeletal muscle. A combined homozygosity mapping and bioinformatics approach revealed a total of 50Mb of significant regions of homozygosity, encompassing 338 genes. Candidate genes were selected for sequence analysis via a bioinformatics approach including data from the Mitocarta database.

*Results and Conclusions:* Our approach led to the identification of a homozygous mutation in *FOXRED1*, a novel molecular chaperone which promotes the correct assembly of mitochondrial complex I. Silencing of *FOXRED1* in human fibroblasts resulted in reduced complex I steady-state levels and activity, whilst lentiviral-mediated *FOXRED1* transgene expression rescued complex I deficiency in the patient fibroblasts.

This FAD-dependent oxidoreductase, which has never previously been associated with human disease, is now shown to be a complex I-specific assembly factor. The discovery of the c.1054C>T; p.R352W mutation in the *FOXRED1* gene is a further contribution towards resolving the complex puzzle of the genetic basis of human mitochondrial disease.

## Poster 48

### **Role of non-muscle myosin heavy chain IIA and $\beta$ -actin in mitochondrial DNA maintenance and segregation**

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Mitochondria contain their own DNA, which contributes essential proteins for aerobic energy production. Mutant mitochondrial DNA therefore precipitates an energy crisis, resulting in a diverse set of human diseases. Many patients with neuromuscular disease caused by mitochondrial dysfunction carry a mixture of mutant and normal mitochondrial DNA. We are studying mitochondrial DNA maintenance and segregation as these processes are predicted to be key determinants of the level of mutant mitochondrial DNA in cells and tissues. Recently we have identified two cytoskeletal proteins tightly associated with mitochondrial DNA, inside mitochondria: non-muscle myosin heavy chain IIA and  $\beta$ -actin. Transient gene-silencing of *MYH9* (encoding non-muscle myosin heavy chain IIA), or the closely related *MYH10* gene (encoding non-muscle myosin heavy chain IIB), altered the topology and increased the copy number of mitochondrial DNA, whereas, genetic ablation of mouse non-muscle myosin IIB was associated with a 60 % decrease in mitochondrial DNA copy number, compared to control cells. Gene-silencing of  $\beta$ -actin also affected mitochondrial DNA copy number and mitochondrial organization. Collectively, these results implicate the actomyosin cytoskeleton in human mitochondrial DNA maintenance; accordingly, these proteins need to be investigated further to understand their roles in mitochondrial diseases.

## Poster 49

### **Analysis of mitochondrial DNA mutant loads in oocytes & preimplantation embryos for the 14709T>C & 14487T>C mtDNA mutations by pyrosequencing**

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Mitochondrial DNA (mtDNA) is strictly maternally inherited. Therefore a woman harboring a pathogenic mtDNA mutation has the potential to transmit a variable amount of mutant mtDNA to each of her offspring, due to the phenomenon of heteroplasmy; the co-existence of wild-type and mutant mtDNA in the same cell. Matters are further complicated by a lack of understanding regarding how these mutant mtDNA species segregate among tissues in the developing embryo. Therefore at risk couples planning a family often pursue genetic counseling, to determine their likelihood of conceiving a child severely affected by an mtDNA disorder.

Here we have examined the mutant load for two mtDNA mutations; 14709T>C and 14487T>C in both oocytes and cleavage stage embryos for two patients undergoing *in vitro fertilization* (IVF) procedures. Mutant load analysis performed on individual blastomeres for the 14709T>C mtDNA mutation revealed overall heteroplasmy levels which ranged from as low as 35% to as high as 45%. For the 14487T>C mutation, low levels of heteroplasmy (<10%) were detected in all individual blastomeres analysed, for a total of five separate embryos. We also examined the mtDNA copy number in individual blastomeres and found the number of mtDNA molecules to be similar between cells of the same embryo.

In summary these results successfully demonstrate the even segregation of mutant mtDNA species between cells of the same embryo, for these two specific mtDNA mutations. Furthermore they reveal the existence of similar levels of heteroplasmy between embryos sampled from the same patient. This therefore provides invaluable data for use in genetic counseling for these two mtDNA mutations (14709T>C and 14487T>C).

## Poster 50

### **Manipulation of human abnormally fertilized pronuclear stage zygotes following**

**vitrification** Laura Irving, L. Craven, M. Herbert & D.M. Turnbull, *Mitochondrial Research Group,*

So far there has been limited success in developing effective treatments for mtDNA disease therefore as mtDNA is transmitted maternally, nuclear transfer techniques have been suggested as a means by which to prevent transmission of mtDNA disease to future offspring. Studies conducted so far in the lab using human abnormally fertilized multipronuclear stage zygotes have demonstrated the feasibility of such techniques for preventing transmission (Craven *et al*, 2010). However it has previously been shown that abnormally fertilised embryos have a limited potential for development. Therefore we propose to create normally fertilized (2PN) embryos by activating donated metaphase II stage oocytes, received by donors to determine the potential of the pronuclear transfer in normally fertilized embryos. However it is possible that we will be unable to synchronize each of the donors cycles therefore we require a means by which to cryopreserve these oocytes following activation.

Here we sought to determine the feasibility of the novel cryopreservation technique known as vitrification as a means by which to store human pronuclear stage zygotes. So far we have currently vitrified a number (n=72) of abnormally fertilised multipronuclear PN stage embryos, using the McGill cryoleaf. Here post-thaw survival rates were determined and embryo morphology assessed using a pre-determined criteria. Embryos were then either cultured to assess developmental potential following vitrification or considered for manipulation procedures. Those embryos which we did attempt to manipulate went onto to develop to cleavage stage embryos, demonstrating the feasibility of vitrification as a means by which to successfully store embryos.

#### Poster 51

##### **Exercise training in patients with mitochondrial myopathy: the analysis of COX-intermediate fibres**

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Mitochondrial myopathies are a group of progressive muscle disorders caused by mutations in the mitochondrial genome (mtDNA). There has been significant progress in the management and diagnosis of patients, however there still remains a need for effective treatments. One proposed treatment is resistance exercise training which has been shown to induce physiological improvements in muscle function and lead to changes in mtDNA mutation load (Murphy *et al*. *Brain* 2008; **131**: 2832-2840).

During exercise training studies of patients with mitochondrial myopathy it was found that the analysis of patient biopsies during this study was complicated by the presence of what we have called "intermediate fibres", which proved difficult to categorise in terms of their mitochondrial enzyme activity since they seem to have partial histochemical cytochrome c oxidase (COX) activity. Given these fibres appear neither fully COX-deficient or COX-positive, it is important to class these intermediate fibres separately rather than including them within the generic description of fibres exhibiting COX deficiency, because variations in the levels of these COX-intermediate muscle fibres could indicate subtle and early stages in a response of the muscle biochemical phenotype to exercise. We have therefore further characterised these fibres by performing densitometric assessment of COX activity within each fibre, and correlating this to mtDNA mutation load. Our data highlight the limitations of the sequential COX/SDH reaction when applying downstream molecular testing including real time PCR protocols.

#### Poster 52

##### **Diabetes is a risk factor for hypertension in adults with the m.3243A>G mitochondrial DNA mutation**

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Background: Diabetes mellitus is a frequent clinical manifestation of mitochondrial disease due to the m.3243A>G mutation.<sup>1</sup> The prevalence of hypertension in patients with diabetes mellitus is approximately 1.5-2.0 times greater than in matched non-diabetic individuals in the general population.<sup>2</sup> Elevated body mass index (BMI) is associated with an increased risk of developing hypertension, yet the prevalence of these diagnoses in patients with mitochondrial disease is unknown.

Aims: To determine the frequency of hypertension and abnormal body mass index in adult patients with mitochondrial disease due to the m.3243A>G mutation.

Methods: Patients were identified from the Medical Research Council Centre for Neuromuscular Diseases Mitochondrial Diseases Patient Cohort Study UK. BMI and non-invasive blood pressure (NIBP) were recorded in all patients attending out-patients clinic between January 2009 and December 2010. Hypertension was defined as a persistent systolic blood pressure  $\geq 140$ mmHg or diastolic blood pressure  $\geq 90$ mmHg. BMI was calculated as weight (kg) / [height (m)]<sup>2</sup>.

Results:

Complete data on 29 patients was available (13 male, 16 female). Hypertension was present in 5 unrelated patients: BMI <20 = 1 (female), BMI 20-25 = 3 (2 male, 1 female), BMI >25 = 1 (female). 8 patients had diabetes or impaired glucose tolerance; 4 of these had co-existent hypertension (BMI <20 = 1, BMI 20-25 = 2, BMI >25 = 1).

Conclusion: Hypertension is no more common in patients with the m.3243A>G mutation than the general population, and does not correlate with BMI. However, diabetes is an additional risk factor in this cohort. Given the existence of evidence-based therapies that lower blood pressure, screening for cardiovascular risk factors is advisable in all patients with the m.3243A>G mutation, particularly those with co-existent diabetes.

1. Whittaker, R.G., *et al.* Prevalence and progression of diabetes in mitochondrial disease. *Diabetologia* **50**, 2085-2089 (2007).
2. Simonson, D.C. Etiology and prevalence of hypertension in diabetic patients. *Diabetes Care* **11**, 821-827 (1988).

### Poster 53

#### **Diabetes is not a common feature in children with mtDNA disease**

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Background Mutations in mitochondrial (mtDNA) cause a diverse spectrum of clinical features in children and young adults, as a result of defective oxidative phosphorylation. Once considered primarily a neurological disease, there is clear evidence that these disorders can cause multi-system problems and result in significant morbidity. Diabetes is a common finding in adult patients with mtDNA disease but is less well documented in children and adolescents suggesting a slowly progressive course or trigger in later life.

Aims: To determine the incidence of diabetes in children and young adults with mtDNA disease

Methods: Patients were identified using the Medical Research Council Centre for Neuromuscular Diseases Mitochondrial Disease Patient Cohort Study UK. Children were defined as having diabetes if they were on insulin, or other medication for glycaemic control, or had a HbA1C >8% and a random glucose >11.1mmol/L but not yet started on treatment.

Results: Information on 24 children and adolescents with mtDNA disease was reviewed (age range 2 yrs 10 months to 21yrs). 1 patient had evidence of diabetes requiring treatment with insulin (male, aged 10yrs 7 months, large scale single deletion). No patients exhibited impaired glucose tolerance.

Conclusions: It is unusual for diabetes to feature early in mtDNA disease. Screening for impaired glucose tolerance should therefore occur regularly to detect diabetes early, institute treatment and aim to prevent secondary complications. Further work to identify the reason for impaired glucose tolerance, and the onset of diabetes, in patients with mtDNA mutations is required.

## Poster 54

### **Kearns-Sayre syndrome caused by defective R1/p53R2 assembly**

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**Background:** Mutations in *RRM2B* encoding ribonucleotide reductase (RNR) p53R2 subunit usually cause paediatric-onset mitochondrial disease associated with mitochondrial DNA (mtDNA) depletion. The importance of RNR dysfunction in adult mitochondrial disease is unclear. We report *RRM2B* mutation frequency in adults with multiple mtDNA deletions, and examine RNR assembly in a patient with Kearns-Sayre syndrome (KSS) caused by two novel *RRM2B* mutations.

**Methods:** Fifty adult patients with multiple mtDNA deletions in skeletal muscle were studied. DNA sequencing of *RRM2B* was performed in patients without mutations in mtDNA maintenance genes *POLG* and *C10orf2*. RNR protein was studied using Western blot and Blue-native gel electrophoresis (BN-PAGE).

**Results:** Four percent (two unrelated cases) of this adult cohort harboured *RRM2B* mutations. Patient 1 had KSS and two novel missense mutations: c.122G>A; p.Arg41Gln and c.391G>A; p.Glu131Lys. BN-PAGE demonstrated reduced heterotetrameric R1/p53R2 RNR levels compared to controls, despite normal steady state p53R2 levels on Western blot, suggesting failed assembly of functional RNR as a potential disease mechanism. Patient 2 had late-onset progressive external ophthalmoplegia and fatigue. We identified a heterozygous deletion c.253\_255delGAG; p.Glu85del. Muscle histology in both cases revealed significant numbers of necrotic muscle fibres, possibly indicating enhanced apoptotic cell death.

**Conclusions:** These data indicate 4% of adult mitochondrial disease with multiple deletions is caused by RNR dysfunction. KSS has not previously been linked to a nuclear gene defect. We provide evidence that disease pathogenesis may be caused by defective RNR assembly. *RRM2B* screening should be considered early in the differential diagnosis of adults with multiple mtDNA deletions.

## Poster 55

### **A3243G – more than just MELAS!**

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**Background** The m.3243A>G transversion in *MTTL1* is one of the most common pathogenic mtDNA mutations and may lead to several clinical phenotypes including Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS), Maternally Inherited Deafness and Diabetes (MIDD) and Chronic Progressive External Ophthalmoplegia (CPEO), although the phenotypic spectrum is usually more diverse. Additional features commonly associated with these syndromes include migraine, bowel dysmotility, and short stature.

Aims To review the phenotypic spectrum of patients harbouring the m.3243A>G mtDNA mutation.

Methods Patients were identified from the Medical Research Council (MRC) Centre for Neuromuscular Diseases Mitochondrial Disease Patient Cohort Study UK. The phenotype of patients harbouring the m.3243A>G mtDNA mutation was analysed by two reviewers.

Results One hundred and eight patient records were reviewed (age range 2 years 10 months to 74 years 1 month). Eight percent patients exhibited the classical MELAS phenotype, 29% had MIDD, 5% had MELAS/MIDD overlap, none had CPEO in isolation, 5% demonstrated a MIDD/CPEO overlap, and 6% CPEO plus syndrome. Eighteen percent of patients suffered with cardiomyopathy and/or other features consistent with mitochondrial disease.

Conclusion The A3243G mtDNA mutation is responsible for a broad phenotypic spectrum. Different clinical syndromes share common features and also exhibit complications not encompassed by their respective acronyms. We feel revision of the current classification system is required to account for this variety and overlap. Central nervous system involvement predominates in adults but usually in combination with at least one other system. Full clinical assessment, including cardiac screening and audiological assessment, is warranted in these patients.

## Poster 56

### Reversible Infantile Respiratory Chain Deficiency is a Genetically Heterogenous Mitochondrial Disease

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A homoplasmic maternally inherited, m.14674T>C mt-tRNA<sup>Glu</sup> mutation was recently identified in Reversible infantile cytochrome c oxidase deficiency, We sought other genetic defects that may give rise to similar presentations.

Patients: The study cohort consists of 8 patients from 7 families with clinical features of infantile reversible cytochrome c oxidase deficiency myopathy.

Methods: We reviewed the diagnostic features, performed biochemical analysis of fibroblasts and molecular genetic analyses of mitochondrial DNA and nuclear-encoded candidate genes.

Results: Presentation was subacute feeding difficulties due to profound hypotonia, and lactic acidosis in infancy. Although recovery was remarkable, a mild myopathy in adulthood was recognized. Histopathological findings in muscle included increased lipid and/or glycogen content, ragged-red and COX negative fibres. Biochemical studies suggested more generalized abnormalities than pure COX deficiency. Clinical improvement was associated with normalization of lactic acidosis and histopathological abnormalities. The m.14674T>C mt-tRNA<sup>Glu</sup> mutation was identified in unrelated 4 families. We also found pathogenic mutations in a related nuclear gene in 2 families. This gene has



previously been associated with mtDNA disease with a different phenotype. In one family the genetic etiology is unknown.

Conclusions: Benign COX deficiency, better described as “Reversible Infantile Respiratory Chain Deficiency”, is genetically heterogeneous. Patients not carrying the m.14674T>C mt-tRNA<sup>Glu</sup> mutation may have mutations in a related gene. Diagnosing this disorder at the molecular level is a significant advance for paediatric neurologists and intensive care paediatricians. It enables them to select children with an excellent prognosis for continuing respiratory support from those with severe mitochondrial presentation in infancy.

#### Poster 57

##### **Why does mitochondrial disease progress? From molecular genetics to patient phenotype**

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Patients with mitochondrial (mt) disease which are due to a disruption of the mtDNA do deteriorate clinically, albeit, to varying degrees. However, it is still unclear how this occurs. It follows that concentrating on a subset of patients where one single genetic defect is known to cause symptoms would aid our understanding of mitochondrial disease progression as a whole. Single large-scale mtDNA deletion disease is a well-described condition where a single genetic defect can manifest differently in individuals. While some patients may present early in life with severe phenotypes: Kearns-Sayre Syndrome, Pearson's Syndrome; other patients will present later on with milder symptoms such as chronic progressive external ophthalmoplegia (CPEO) or myopathy. Here at Newcastle, we are privileged to be able to follow these patients in the Mitochondrial Disease clinic and have their informed consent for data collection on their clinical and genetic status for research purposes. By investigating how and why patients progress, we will be able to develop therapeutic strategies.

The hypothesis we have developed is that the increasing inability of patients to cope with mtDNA deletion load due to reducing wildtype mtDNA copy number with age leads to a progressive biochemical defect seen in muscle biopsies with time, and this subsequently correlates with their phenotypic features, namely, myopathy.

The aim of our research is to investigate this hypothesis in order to start unravelling some of the complexities surrounding mitochondrial disease.

#### Poster 58

##### **Mitochondrial DNA Mutations in Satellite Cells**

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Mitochondrial myopathies are a group of progressive muscle disorders caused by mutations in the mitochondrial genome (mtDNA) for which there is no effective treatment. Culturing of myoblasts from patients harbouring some sporadically occurring mtDNA diseases has suggested that mutations may be absent in satellite cells. The activation of satellite cells and subsequent repair of muscle fibres may favourably shift the balance of deleted to wild-type mtDNA, thereby decreasing mtDNA mutation load in affected muscle. We have investigated satellite cells from patients with mitochondrial myopathy due sporadic mtDNA deletions to determine if they will benefit from attempts to shift the balance of wild-type and mutated mtDNA in their muscles using high intensity resistance training.

Using Fluorescently Activated Cell Sorting to isolate satellite cells, and Real Time PCR assays, we have detected mtDNA deletions in the satellite cells of all seven patients investigated at level similar to mature muscle. In most of these patients the mtDNA deletions are lost during the culturing of their myoblasts. In some patients, however, the mutation is maintained, although there is a gradual

decline in mutation load as the myoblasts head towards differentiation. We have hypothesised that this difference between patients in the maintenance or loss of mutations in their myoblasts could be attributable to an mtDNA bottleneck effect.

Previous research has shown an improvement in mitochondrial activity in muscle fibres after resistance training, and current research suggests this could be attributed to the regeneration of respiratory abnormal fibres. The loss of mtDNA deletions early on in myoblasts, or the gradual decline in mutation levels as the cells head towards differentiation, could explain these findings.

#### **Poster 59**

##### **Mitochondrial respiratory chain enzyme deficiency expressed during muscle development**

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We studied myoblast cell cultures from four unrelated paediatric patients with marked mitochondrial respiratory chain enzyme deficiencies in skeletal muscle, but with no apparent mitochondrial DNA abnormalities. Despite the clear enzyme defects in muscle tissue, biochemical assays revealed only mildly affected respiratory chain enzyme activities in a myoblast culture from one patient, while the activities were normal in myoblast cultures from the other three patients. Immunoblot analysis of blue native gels and cytochemical staining of the myoblast cultures corroborated the results of the biochemical assays. Myotubes, however, were clearly affected in all four patient cultures. Myotubes derived from the patient myoblast culture with mild respiratory chain enzyme deficiency showed no cytochrome-c oxidase staining and did not fully develop. Myotubes derived from the three patient myoblast cultures with normal respiratory chain enzyme activity did fully develop but showed uneven cytochrome-c oxidase staining. In both patient and control myotubes, steady-state levels of respiratory chain enzyme subunits were higher than in myoblasts; however, in contrast to control myotubes, patient myotubes contained swollen and unevenly distributed mitochondria. The life span of patient myotubes was dramatically shorter than that of control myotubes. Our results suggest that respiratory chain enzyme defects seen in skeletal muscle biopsies from these patients may be due to, or enhanced by, a failure of normal mitochondrial biogenesis during muscle development.

#### **Poster 60**

##### **Respiratory chain complex I deficiency caused by mitochondrial DNA mutations**

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Mitochondrial respiratory chain defects are associated with a wide range of clinical presentations and may be caused by mutations in either the nuclear or mitochondrial genome (mtDNA). Isolated complex I deficiency is the most frequently observed enzyme defect in mitochondrial disease, particularly in the paediatric population where family history is often consistent with sporadic or autosomal recessive inheritance, implicating an underlying nuclear genetic cause. Although a number of recurrent, pathogenic mtDNA mutations have been described, historically these have been perceived as rare causes of paediatric complex I deficiency.

We reviewed the clinical and genetic findings in a large cohort of 109 paediatric patients with isolated complex I deficiency referred to a single national reference laboratory for diagnostic testing. Pathogenic mtDNA mutations were found in 29/101 probands (29%), 21 in *MTND* genes encoding structural complex I subunits and 8 in mt-tRNA genes. Nuclear gene defects were inferred in 38/101 (38%) probands based on cell hybrid studies, mtDNA sequencing or mutation analysis (nuclear gene

mutations were identified in 22 probands). Leigh or Leigh-like disease was the most common clinical presentation in both mtDNA and nuclear genetic defects. Median age of onset was higher in mtDNA patients (12 months) than patients with a nuclear gene defect (3 months), although considerable overlap existed with onset ranging from 0m to >60m in both groups. Our data confirm that pathogenic mtDNA mutations are a significant cause of complex I deficiency in children and as such sequencing the entire mitochondrial genome is a key step in the diagnostic algorithm to elucidate the underlying molecular genetic abnormality.

## **Congenital Myopathies and Limb Girdle Myopathies**

### **Poster 61**

#### **The spectrum of genetic defects responsible for congenital fibre type disproportion**

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The term congenital fibre type disproportion (CFTD) was originally assigned to muscle biopsies showing type I fibres at least 12% smaller than type II fibres (later revised to 25%) with no other structural defects and a fairly consistent clinical picture of congenital myopathy. Although debatable, the concept of CFTD has been gradually extended as a clinical and pathological condition. Genetic base of CFTD was unknown until 2004 when *ACTA1* was linked to CFTD. Subsequently three additional genes have been found to be responsible, *TPM3*, *SEPN1* and *RYR1*.

We reviewed 5 muscle biopsies with confirmed mutations (2 *ACTA1*, 2 *TPM3* and 1 *RYR1*), all showing typical CFTD, type I atrophy and type II hypertrophy. The fibre size differences range from 21% to 58% with no other structural changes. Although most type I fibres appeared smaller, myosin immunolabelling revealed some larger slow fibres, several of which co-expressed fast myosin. No pathological features correlate gene defects are found in this study.

Although all of our cases fell into the category for CFTD some co-expression of myosin isoforms was common. No distinct pathological features were present to guide specific molecular analysis. Therefore pathology alone cannot predict the genotype. The current view on frequency of genetic defects associated with CFTD is *TPM3* > *RYR1* > *ACTA1* and *SEPN1*. To date genetic defects have not been found in 50% of cases with CFTD (Clarke et al., 2010 Human Mutation) and it is likely that more genes associated with this pathology will be identified.

### **Poster 62**

#### **The Identification of a Viable Outcome Measure in the Collagen VI Myopathies Promotes Progress Toward Clinical Trials**

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Background: The optimization of standard of care and the development of potential therapies for the collagen VI myopathies [Ullrich congenital muscular dystrophy (UCMD), Bethlem myopathy (BM) and intermediate phenotypes] make the identification of outcome measures essential. Significant, progressive joint contractures complicate assessments of motor function and strength in this population. In those patients with UCMD and intermediate phenotypes, respiratory involvement is highly relevant to disease progression. Forced vital capacity (FVC) is a quantitative measure of respiratory function which can be obtained regardless of the severity of joint contractures.

Aim: An international collaboration to collect and study longitudinal FVC data in the collagen VI myopathies.

Methods: Retrospective chart reviews of patients with molecularly confirmed or biochemically confirmed diagnoses of collagen VI myopathies were performed at 9 neuromuscular centres: United States (2), United Kingdom (2), France (1), Italy (2), Belgium (1) and Australia (1).

Results: A total of 461 FVC measurements obtained in 214 patients were analyzed. All patients at the moderate to severe end of the phenotypic spectrum demonstrated invariable decline of FVC from age 7 years with the onset of nighttime BiPAP use in 44 patients at an average age of 11.28 +/- 4.31 years. Patients at the mildest end of the phenotypic spectrum did not demonstrate a clear pattern of decline in respiratory function.

Conclusions: Forced vital capacity is a viable outcome measure for patients at the moderate to severe end of the collagen VI myopathy phenotypic spectrum, a finding which should inform optimal surveillance and clinical trial planning.

### Poster 63

#### **A founder mutation in Anoctamin 5 is a major cause of Limb Girdle Muscular Dystrophy**

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The limb-girdle muscular dystrophies (LGMDs) show wide genetic and clinical heterogeneity. Mutations in the *ANO5* gene, which encodes a putative calcium-activated chloride channel of the Anoctamin family, were found in previously identified disorders, LGMD2L and non-dysferlin Miyoshi muscular dystrophy (MMD3). We screened a candidate group of 64 patients and found the truncating mutation, c.191dupA in exon 5 in 20 patients, homozygously in 15 and in compound heterozygosity in the rest. An intragenic SNP and an extragenic microsatellite marker are in linkage disequilibrium with the mutation, suggesting a founder effect in the Northern European population. This has allowed further definition of the clinical phenotype. Patients show adult onset proximal lower limb weakness with highly raised CK values (average 4500 IU/L) and frequent muscle atrophy and asymmetry of muscle involvement. Onset varies from the early 20s to 50s and the weakness is generally slowly progressive, with most patients remaining ambulant for several decades. Distal presentation is much less common but a milder degree of distal lower limb weakness is often observed. Upper limb strength is only mildly affected and cardiac and respiratory function is normal. Females appear less frequently affected. In the North of England population we estimate a minimum prevalence of 0.27/100 000, twice as common as dysferlinopathy. We suggest that mutations in *ANO5* represent a relatively common cause of adult onset muscular dystrophy with high CK and that mutation screening, particularly of the common mutation c.191dupA, should be an early step in the diagnostic algorithm of adult LGMD patients.

## Poster 64

### Neutral lipid storage myopathy due to *PNPLA2* mutations may respond to beta-adrenergic treatment

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**Background:** Neutral lipid storage disease is caused by mutations in the *CGI-58* or the *PNPLA2* genes. Lipid storage can be detected in various cell types including blood granulocytes. While *CGI-58* mutations are associated with Chanarin-Dorfman syndrome, a condition characterized by lipid storage and skin involvement (ichthyosis), mutations in the patatin-like phospholipase domain-containing protein 2 (*PNPLA2*) were reported with skeletal and cardiac muscle disease only.

**Methods:** We describe in detail clinical, myopathological and MRI findings of 6 patients with different recessive *PNPLA2* mutations. Pulse-chase labeling of control and patient cells with supplementation of clenbuterol, salmeterol and dexamethasone was performed *in vitro*. **Results:** The patients share a recognizable clinical phenotype with prominent shoulder girdle weakness, mild pelvic girdle and distal muscle weakness with highly elevated CK and cardiomyopathy developing at later stages. Muscle histology invariably reveals massive accumulation of lipid droplets. New muscle or whole-body MRI techniques may assist diagnosis and may become a useful tool to quantify intramuscular lipid storage. Activation of hormone-sensitive lipase by beta-adrenergic substances such as clenbuterol appears to bypass the enzymatic block in *PNPLA2*-deficient patient cells *in vitro*. **Conclusions:** *PNPLA2* deficiency is a slowly progressive myopathy with onset around the third decade. The diagnosis can be made by staining for Jordans' anomaly in peripheral blood smear. Cardiac involvement is relatively common at a later stage. Muscle MRI may detect increased lipid in a characteristic distribution, which could be used for monitoring disease progression. Beta-adrenergic agents may be beneficial in improving triacylglycerol breakdown in patients with *PNPLA2* mutations.

## Poster 65

### Whole genome analysis in a family with dominant muscle disease

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Major technical advances in next generation sequencing platforms have resulted in increasing availability of whole genome analysis. To date this is principally available as a research tool although its transition into clinical diagnostics will likely follow with potentially major implications for medical practice. Commercial availability of whole genome analysis directly to patients has now started and we report a family who followed this route. The index case was born uneventfully at term and had normal motor milestones. He could never run and from mid-teens developed proximal upper and lower limb weakness. On examination age 33 there were mild biceps contractures and proximal upper and lower limb weakness MRC 4/5. His mother gave an identical history and remains ambulant with one stick age 60. CK was 354 IU/L (reference range 26-140 IU/L) and muscle biopsy non-specifically mildly myopathic. Lamin A/C gene analysis was normal. The family was counselled as for a dominant muscle disease and advised further gene testing would be undertaken as it became available. He was lost to follow-up. Six years later the family represented having commercially obtained whole genome sequence data for the index case, his mother and father and his wife. The commercial analysis had been undertaken principally to provide risk evaluation for common vascular, degenerative and oncological diseases. We had analysed the whole genome data using a bioinformatic filtering technique to identify non-synonymous variants in muscle expressed genes. We identified such variants in 17 genes known to associate with muscle disease. Correlations with muscle immunohistochemistry and MRI imaging patterns allowed us to identify a

pathogenic variant in exon 3 of COL6A1 (N1 domain): c.347G>A; p.Ser116Asn. This was shared by his affected mother but not his unaffected father. MRI of lower limb muscles demonstrated a pattern highly characteristic and specific for a COL1A6 disorder. The diagnosis of dominant Bethlem myopathy was therefore confirmed. This family exemplifies next-generation sequencing as a diagnostic tool and highlights its current commercial availability. Careful correlation of clinical, muscle histochemical and MRI data with genetic findings and close collaboration between physician and bioinformatician was essential in making the diagnosis. As cost continues to decline it seems probable that an increasing number of patients will pursue whole genome analysis independently and this will present challenges for their treating physicians.

## Poster 66

### Functional investigation of beta-tropomyosin mutations that cause congenital skeletal myopathies

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Congenital myopathies are a group of inherited muscle diseases which result in abnormalities of muscle structure. The majority of identified disease genes encode contractile proteins and different mutations in certain genes can cause distinct diseases, giving the possibility of analysing the molecular basis of genotype: phenotype correlations. One such gene is *TPM2* which encodes beta-tropomyosin, a protein involved in the Ca<sup>2+</sup>-regulation of contractility. Different *TPM2* mutations cause four distinct phenotypes: distal arthrogyrosis types 1 and 2A (DA), nemaline myopathy (characterised by the formation of nemaline rods) (NM), cap disease (defined by protein aggregates – “caps” - on the periphery of muscle fibres beneath the sarcolemma), and muscle weakness with the presence of both nemaline rods and “caps”. In order to analyse how each of these mutations affect contractile function and thin filament stability, recombinant wild type human beta-tropomyosin and six mutants have been produced and their behaviour compared with wild type in a series of functional assays. DA mutant tropomyosins either increase actomyosin ATPase at all Ca<sup>2+</sup> concentrations or cause an increase in Ca<sup>2+</sup>-sensitivity of regulation, suggesting a resultant hypercontractile muscle. The mutants causing NM and/or cap disease appear to give a more varied molecular phenotype, suggesting either dramatically lowered actin binding (and hence filament instability) or normal actin binding but with regulatory properties suggesting reduced contractility. We are currently assessing how these mutants affect contractility in situ in demembranated muscle fibres.

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## Poster 67

### SEPN1 related myopathies: Clinical course in a large cohort of patients

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Objective: To describe the clinical course and genotype/ phenotype correlations in a large cohort of patients affected by selenoprotein related myopathy (SEPN1-RM) due to *selenoprotein N1* gene mutations.

Methods: this is a retrospective cross-sectional study of forty-one patients aged 1-60 years. Clinical data were collected by case note review.

Results: The mean age of onset was 2.7 years, ranging from birth to second decade of life. The majority of the patients (85%) remain fully ambulant and independent, however one lost ambulation at age 5 years and another in his late fifties, while another four cases needed wheelchair for long distances. The mean age of starting nocturnal non-invasive ventilation was 14.3 years. One child required full time BIPAP when aged one year while in two cases non-invasive ventilation was started at 33 years. Two patients died from respiratory failure at the age of 10 and 22 years. The mean age of scoliosis onset was 10 years, in most cases preceded by spinal rigidity. Fourteen patients had a successful spinal surgery (mean age 13.9 years). Twenty-one were underweight; however overt feeding difficulties were not a feature.

Conclusions: This study describes the largest population affected by SEPN1-RM reported so far showing that the spectrum of severity is wider than previously reported. Respiratory insufficiency generally develops by 14 years but may occur as early as in infancy or not until the fourth decade. Motor abilities remain essentially static over time even in patients with early presentation. Most adult patients remain ambulant and fully employed.

#### Poster 68

#### The pathological spectrum associated with mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene

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Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are associated with dominantly and recessively inherited congenital myopathies and the malignant hyperthermia susceptibility trait. Muscle pathology in combination with clinical and muscle MRI assessment can help direct molecular analysis. We illustrate here the most useful pathological features in muscle biopsies that aid diagnosis.

A common feature is an increase in internal nuclei, often multiple and sometimes central, resembling centronuclear myopathy. Predominance or uniformity of type 1 fibres is also common. Small type 1 fibres with large type 2 fibres, resembling fibre type disproportion can occur, sometimes in association with cores or increased internal nuclei. Connective and adipose tissue can be extensive, causing diagnostic confusion with congenital muscular dystrophy, but necrosis is not typical. Cores devoid of mitochondria are of varying size, number and position. Sometimes only subtle unevenness, or normal staining is seen. Cores show variable accumulation of proteins, including desmin, myotilin,  $\alpha$ -actinin,  $\gamma$ -filamin,  $\alpha$ B-crystallin, SERCA 1 and 2, RyR1, calsequestrin but this is not usually of diagnostic help. Immunolabelling of myosin isoforms confirms the predominance or uniformity of fibres with slow myosin and reveals a few hybrid fibres with more than one isoform. Very small fibres with neonatal and fast myosin are sometimes present.

The pathological spectrum associated with *RYR1* mutations continues to expand, and may even be minimal. The most indicative features are central nuclei, type 1/slow fibre uniformity or predominance, core-like lesions of varying size or unevenness of oxidative stains, and very small fibres expressing neonatal myosin. Clinical correlations are essential.

## **Inclusion Body Myositis and Myofibrillar Myopathies**

### **Poster 69**

#### **Investigating the effects of pharmacological up-regulation of the heat shock response on protein degradation pathways in an *in-vitro* model of sporadic inclusion body myositis**

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Sporadic inclusion body myositis (sIBM) is the commonest acquired muscle disease affecting adults over the age of 50. Although the aetiology of this disease remains unclear, there is evidence for both inflammatory and myodegenerative processes in sIBM muscle pathology. In particular, abnormal protein aggregation is a characteristic feature of affected muscle, with inclusions incorporating several well studied proteins including amyloid-beta precursor protein ( $\beta$ -APP), amyloid-beta ( $A\beta$ ), phosphorylated tau and heat shock proteins (HSPs) among many others.

The heat shock response (HSR) is involved both in the regulation of normal protein folding and the disaggregation of aggregated proteins. Up-regulation of the HSR and the subsequent elevation in HSP expression has been investigated as a potential therapeutic strategy in a number of cellular and animal models of neurodegenerative disease.

In this study, we examined the effects of pharmacological up-regulation of the HSR in an *in vitro* model of sIBM. Using primary muscle cultures derived from neonatal rats, we found that transfection with  $\beta$ -APP results in increased cytotoxicity and proteasome dysfunction. Up-regulation of the HSR was found to significantly improve cell survival although this was not accompanied by an improvement in proteasome function. These findings suggest that the cytoprotective effects of increased HSP expression occur upstream to the proteasome degradation pathway. The expression of p62, an ubiquitinated-polypeptide shuttle protein, was also investigated and found to aggregate in the nucleus and cytoplasm of transfected muscle cells. Since p62 transports proteins for degradation via both the proteasomal and lysosomal pathways, we are currently investigating the effects of  $\beta$ -APP transfection and pharmacological up-regulation of the HSR on autophagy in this *in vitro* model of sIBM.

### **Poster 70**

#### **Inclusion body myositis: a diagnostic challenge**

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Background: The currently accepted diagnostic criteria for inclusion body myositis (IBM), first published in 1995, comprise pathological and clinical elements. The pathological features being endomysial inflammation with invasion of intact fibres, rimmed vacuoles and either amyloid or tubulofilamentous inclusions; all of these must be present for a definite diagnosis. However, these features are not specific to IBM and furthermore in many clinically typical cases one or more may be absent. Recently a variety of proteins more commonly associated with neurodegenerative diseases have been found to accumulate in muscle fibres in IBM. The benefit of immunohistochemical staining for these proteins in the diagnosis of IBM is unknown. We propose that these protein accumulations may assist in the diagnosis of IBM and help in differentiating it from other myopathies.

Aims: To identify the most sensitive immunohistochemical techniques available to UK diagnostic pathology laboratories of detecting such protein inclusions and to determine their sensitivity and specificity for the diagnosis of IBM.

Methods: We identified 6 cases of pathologically definite IBM according to the current criteria and 6 normal control cases. All cases had been assessed clinically and had undergone a biopsy at the National Hospital for Neurology and Neurosurgery, Queen Square, London. A number of



neurodegenerative proteins and inflammatory markers were identified that may be of diagnostic significance. Antibodies to these proteins were optimised using control tissue known to contain the protein of interest.

Initial results: Protein inclusions found in IBM were labelled with an antibody to p62, an adapter protein which binds ubiquitin and regulates signalling cascades through ubiquitination. These inclusions were not found in the normal controls. Further work to quantify the abnormalities in IBM and disease controls will be undertaken before any of these markers can be recommended for diagnostic use.

#### **Poster 71**

##### **A randomised, double-blinded, placebo-controlled pilot study assessing the safety and tolerability of Arimoclomol in sporadic Inclusion Body Myositis (IBM)**

P. Machado<sup>1</sup>, A. Miller<sup>1</sup>, M. Parton<sup>2</sup>, L. Dewar<sup>2</sup>, J. Holton<sup>3</sup>, M. Dimachkie\*, L. Herbelin\*, L. Greensmith<sup>1</sup>, R. Barohn\*, M. Hanna<sup>1</sup> <sup>1</sup>MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK, <sup>2</sup>National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK, <sup>3</sup>UCL Institute of Neurology, Queen Square, London WC1N 3BG, \*The University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA

Background: Arimoclomol is an investigational drug that prolongs the activity of the transcription factor, Heat Shock Factor-1 (HSF-1), which amplifies heat shock protein (HSP) gene expression. HSPs have been shown to attenuate protein misfolding and aggregation promoting cellular defences against such processes. They have also been shown to dampen the inflammatory response, via inhibition of the proinflammatory transcription factor NFκB.

Objective: To make the first assessment of a new therapeutic compound - Arimoclomol - in IBM.

Methods: Randomised double blind placebo controlled study (2:1), with two recruitment sites: London, UK (12 patients) and Kansas, US (12 patients). Patients have to fulfil Griggs criteria for definite or probable IBM and age must be >50 years. The primary outcome is adverse event reporting (safety and tolerability). Secondary outcomes are the assessment of changes in the levels of HSPs and pathological changes in muscle tissue, measures of muscle strength (manual muscle testing, fixed myometry), muscle mass measures (DEXA) and a functional rating scale (IBM-FRS). Results: The US site has finished recruitment. The UK site has recruited 9 patients so far (6 males and 3 females, mean age 62 years [standard deviation=5.8]). We expect to finish recruitment by the end of January 2011.

Conclusion: As a pilot study, we seek to answer fundamental questions about Arimoclomol's safety and tolerability and the effect of Arimoclomol on muscle affected by IBM. Since IBM is without any established treatment, this is a particularly important study in the field of muscle disease.

#### **Poster 72**

##### **The Effects of Arimoclomol on Pathological Outcome Measures of Inclusion Body Myositis *in vitro***

Adrian Miller<sup>1,2</sup>, Mhoriam Ahmed<sup>1,2</sup>, Mike Hanna<sup>1</sup>, Linda Greensmith<sup>1,2</sup>

<sup>1</sup>MRC Centre for Neuromuscular Diseases, Queen Square, London WC1N 3BG, UK; <sup>2</sup>Sobell Department of Motor Neuroscience and Movement Disorders, Queen Square, London WC1N 3BG, UK Sporadic inclusion body myositis (IBM) is the commonest myopathy acquired after 50 years of age and causes significant disability through progressive limb weakness and dysphagia. Its pathogenesis appears to combine several interacting inflammatory and degenerative processes. No effective treatment exists despite several clinical trials of immunotherapies.

Through the over-expression of β-Amyloid Precursor Protein or exposure to the inflammatory mediators IL1β, TNFα and IFNγ, we induced IBM-like pathology in primary myogenic rat cell cultures. We then examined the effect of treatment with a new compound, Arimoclomol on a selection of quantifiable disease-relevant outcome measures. Arimoclomol is a co-inducer of the transcription factor HSF-1 that drives expression of several key endogenous Heat Shock Proteins (HSPs), notably HSP70 and HSP90 whose cytoprotective actions include degradation of improperly folded proteins and dampening of pro-inflammatory signals.

Under experimental conditions, cultured myotubes demonstrated several key microscopic features of IBM pathology, including the formation of ubiquitinated intracellular inclusions, increased expression

of Major Histocompatibility Complex Class I and cytoplasmic translocation of TDP-43. We developed quantitative assays of these changes and of several pathogenic processes which are implicated in IBM, including the pro-inflammatory NfκB cascade, endoplasmic reticulum (ER) stress, calcium homeostasis and mitochondrial function. We describe the impact of Arimoclochol treatment on these parameters which, we propose, supports further investigation of Heat Shock Response manipulation as a therapeutic strategy for IBM.

### **Poster 73**

#### **Mitochondrial abnormalities in Inclusion Body Myositis**

Karolina Rygiel, James Miller, Rob Taylor, Doug Turnbull, *Mitochondrial Research Group, IAH, Newcastle University, UK*

As we age our muscles deteriorate and become weaker as a result of a process called sarcopenia. Wasting of skeletal muscles in sarcopenia occurs naturally as a consequence of advancing age. However, a growing percentage of the ageing population suffer from age-related myopathies, which resemble an accelerated sarcopenia. The most commonly diagnosed myopathy in people over 50 is Inclusion Body Myositis (IBM).

IBM is suspected to be an autoimmune disease affecting selected muscle groups and manifesting in progressive weakness. A number of pathologies have been reported in the affected muscle fibres including mitochondrial abnormalities. Our hypothesis is that mitochondrial DNA mutations (mtDNA) accumulate in the muscle fibres and directly contribute to wasting and weakness.

Fifteen IBM muscle biopsies (vastus lateralis) were used for histochemistry, immunohistochemistry and mtDNA analysis. We detected respiratory compromised fibres with COX/SDH reaction and assessed protein expression of selected components of mitochondrial respiratory chain complexes (complex I subunit 19 and 20kDa, complex IV subunit I and IV) and inflammatory cell markers (CD3 and CD68). Single COX+ve and COX-ve cells were then microdissected, lysed and DNA extracted. Real-Time PCR assay and long range PCR were used to detect mtDNA deletions in the samples.

Between 3% and 80% of muscle fibres in individual biopsies were found to be COX deficient (average of 10%). Detection of protein levels showed that, of all the proteins measured, subunits of complex I were affected the earliest by the mtDNA changes. Downregulation of complex I was followed by downregulation of complex IV subunit I together with the loss of COX activity. Molecular analysis of single muscle fibres provided evidence for the presence of multiple mitochondrial deletions in some, but not all of COX-ve fibres. Semi-quantitative analysis of lymphocytes and macrophages in the tissue revealed a clear correlation between severity of immune infiltration and number of COX-ve fibres. Similar correlation was observed between the level of inflammation and atrophy in the biopsies. Additionally, majority of atrophic fibres were COX deficient and the majority of COX-ve cells were atrophic.

This study has shown that mitochondrial DNA mutations (mostly deletions) exist in the affected muscles. It is highly likely that they are formed in response to inflammation and accumulate as a consequence of clonal expansion. Components of complex I become downregulated first and as the ratio of mutant to wild type mtDNA increases the cell loses COX activity. COX deficient fibres gradually become atrophic and die resulting in muscle mass reduction.

### **MRI in Neuromuscular Disease**

#### **Poster 74**

#### **An MRI study of the effects of metoprolol on *in vivo* cardiac calcium homeostasis**

Alison Blain, Elizabeth Grealley, Steve Laval, Volker Straub, Guy MacGowan *Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK*

We have previously shown (by conductance catheter experiments), that metoprolol, a β-1 selective β-blocker, has divergent effects on the cardiac phenotype of δ-sarcoglycan (δ-SG) and mdx mice (models of LGMD2F and DMD, respectively). While treated mdx mice had improved cardiac

contractility, treated  $\delta$ -SG mice showed a deterioration in cardiac function and a propensity to die when challenged with dobutamine. Treated  $\delta$ -SG showed a marked increase in indicators of active relaxation, suggesting abnormally elevated intracellular calcium levels. We therefore wished to investigate if the strain differences in the response to metoprolol treatment could be attributed to differing myocardial calcium influx, as measured *in vivo* by manganese enhanced MRI. Mice were treated with metoprolol (2.5 mg/kg/day, in drinking water), for 8 weeks starting from an age preceding overt cardiomyopathic changes (8 weeks in  $\delta$ SG mice and 16 weeks in mdx). Left ventricular function and manganese uptake were analysed by MRI. Mice were then recovered and underwent cardiac catheterisation 1-4 days later to obtain contractile indices in the same animals. We report the preliminary results of this study and on a correlational analysis of left ventricular volumes obtained from MRI and catheter experiments. MRI and catheter volumes were highly correlated, however MRI volumes were consistently higher than those obtained by catheter, as has been reported previously.

## Poster 75

### Muscle MRI findings in LGMD2L

Anna Sarkozy<sup>1</sup>, Debbie Hicks<sup>1</sup>, James Miller<sup>2</sup>, Maggie C Walter<sup>3</sup>, Peter Reilich<sup>3</sup>, Aleksandar Radunovic<sup>4</sup>, Sujit S. Vaidya<sup>4</sup>, Hanns Lochmuller<sup>1</sup>, Kate Bushby<sup>1</sup>, Volker Straub<sup>1</sup> 1) *Institute of Human Genetics, Newcastle University, UK.* 2) *Department of Neurology, Royal Victoria Infirmary, Newcastle upon Tyne, UK;* 3) *Friedrich Baur Institute, Ludwig-Maximilians University, Munich, Germany;* 4) *The Royal London Hospital, London, UK*

Recessive mutations in the *ANO5* gene cause limb girdle muscular dystrophy type 2L (LGMD2L). Patients present with a pattern of proximal weakness affecting predominantly the pelvic girdle and leg muscles. Distal leg weakness is less prominent, but can occasionally be one of the first presenting clinical symptoms. To further define the pattern of muscle involvement in LGMD2L, we analyzed the distribution and extent of muscle pathology by MRI in muscles of the pelvis and lower limbs in a cohort of 8 LGMD2L patients. Muscle pathology was evaluated on T1 weighted images. LGMD2L patients show a rather homogenous pattern of muscle involvement by MRI. Both proximal and distal muscles were affected in all individuals and no major differences were noticeable between patients with a more proximal or distal clinical presentation or different *ANO5* gene mutations. Patients at different stages of the disease show a more pronounced posterior involvement of the thighs and lower legs, with major involvement of the adductor magnus, semimembranosus, semitendinosus, medial gastrocnemius and soleus. A moderate involvement of the gluteal muscles was noticeable, while the gracilis, sartorius, biceps femoris and muscles of the lower leg anterior compartment appeared to be least affected. The present findings show that patients with *ANO5* mutations present a pattern of pathology on MRI different from what had been observed in other LGMDs, but similar to patients with LGMD2B.

## Poster 76

### MRI Shows Increased Tibial Nerve Size in CMT1A

CDJ Sinclair, JM Morrow, A Fischmann, MG Hanna, MM Reilly, TA Yousry, X Golay, JS Thornton  
*MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*

**Background:** Enlargement of peripheral nerves is a known feature of inherited and acquired demyelinating peripheral neuropathies. Previous work using MRI has reported enlarged sciatic nerve cross-sectional areas in Charcot-Marie-Tooth disease type 1A (CMT1A) and in chronic inflammatory demyelinating polyneuropathy (CIDP). Enlarged spinal nerve roots in CIDP and enlarged peripheral nerves in other types of CMT have also been reported. Here, we examined the size of the tibial nerve in the lower leg of CMT1A patients with high-resolution MRI.

**Methods:** Both lower limbs of 14 CMT1A patients and 16 healthy controls were imaged with a proton-density weighted 2D-FLASH sequence with 0.78mm in-plane resolution. The left and right tibial nerve in the central lower-leg was identified by a radiologist blinded to the diagnosis and the cross sectional area of each nerve was recorded.

**Results:** The mean  $\pm$  standard deviation nerve areas were  $6.4 \pm 2.3 \text{mm}^2$  (controls) and  $23.8 \pm 11.1 \text{mm}^2$  (CMT1A patients). The CMT1A group nerves were statistically enlarged compared to controls (t-test,  $p < 0.001$ ) and the largest CMT1A nerves were up to 9 times bigger than controls. Further work to quantify peripheral nerve features using MRI may assist in distinguishing different types of inherited or acquired peripheral neuropathy and in monitoring disease activity.

#### **Poster 77**

##### **Improved Magnetization Transfer MRI of Skeletal Muscle in Myopathy and Neuropathy**

CDJ Sinclair, JM Morrow, MG Hanna, MM Reilly, TA Yousry, X Golay, JS Thornton

*MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*

**Background:** Magnetic resonance imaging (MRI) may provide sensitive, non invasive markers of muscle pathology suitable as outcome measures in forthcoming therapeutic trials. Magnetization transfer ratio (MTR) MRI in particular shows promise for quantifying tissue-water changes and fatty degeneration in skeletal muscle. However, sensitivity is compromised by unavoidable instrumental imperfections, in particular MRI scanner radio frequency transmit ( $B_1$ ) field non-uniformity. We hypothesised that post-acquisition  $B_1$  correction would reduce scanner-dependent variance and ultimately improve the correlation of MTR indices with clinical severity.

**Method:** We implemented a method, originally developed for brain MTR imaging, to correct for  $B_1$ -dependent inhomogeneities in skeletal muscle MTR maps by acquiring independent  $B_1$  field maps during the same MRI examination. To test the method, the thigh and calf muscles of 10 patients with inclusion body myositis (IBM), 11 Charcot-Marie-Tooth 1A (CMT1A) patients and 28 healthy volunteers were imaged at 3 Tesla.

**Results:** Histogram analysis of the resulting MTR maps showed that this correction reduced the within-subject and between-subject variation in normal muscle by a factor of 2 in both controls and patients. The mean MTR measured bilaterally in the tibialis anterior muscle of volunteers was  $32.7 \pm 6.3$  p.u. before and  $31.9 \pm 1.5$  p.u. after correction, representing a 4 fold improvement (i.e. decrease in standard deviation)

**Conclusion:** The marked reduction in instrumentally-induced variability was obtained with 5 minutes of additional scan time. The method will increase sensitivity of skeletal muscle MTR to genuine disease processes, and by reducing scanner-dependent, and therefore site-dependent variability, increase the utility of MTR imaging in multi-centre trials.

### **Neuromuscular Databases for Translational Research and Clinical Trials**

#### **Poster 78**

##### **An integrative database for clinical and research studies in neuromuscular diseases**

Thomas Müller<sup>1</sup>, Sebahattin Cirak<sup>1</sup>, Matthew Parton<sup>1</sup>, Michael Lunn<sup>1</sup>, Mike Hanna<sup>1</sup>, Francesco Muntoni<sup>1</sup>

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Our aim was to establish a integrative relational database that stores patient demographics, cross-sectional clinical information, genetic data, biopsy data, biological sample inventory and information regarding available consent to support and facilitate clinical and laboratory research within the MRC Centre for Neuromuscular Diseases. For this, a sustainable and relational database which is regularly updated and integrated in the daily work flow is required. The database will support the biobank including adult and paediatric diagnostic services. The main implementation requirements focus on usability by multiple users with different roles, security and ease of maintenance. Because of the multi-user requirement and the maintenance aspect, a web based database system was favoured. Thus authorised users can access the data without the need to install additional software. To gain access, user accounts are provided with passwords and also the data transmitted over the network is encrypted in transit. The database server is hosted in a secure NHS location, which permits easy centralised maintenance. At the core of the implementation is a SQL database including a framework

that provides the user interface, running on ubiquitous server infrastructure. The consultation and development for the integrative database took place over the course of 12 months carried out by a small team including one database developer funded by the Muscular Dystrophy Campaign (MDC). The integrative database allows collaborators from various units of the MRC Centre to rapidly retrieve, enter and update relevant information and enables them to perform customised queries, which save them valuable time for clinical work and research. The open nature provides a sustainable integrative database system to a wider community and adopts it to their special needs.

#### **Poster 79**

##### **The Natural History of Sporadic Inclusion Body Myositis: Development of an Electronic Database IBMnet**

A Miller<sup>1</sup>, P Machado<sup>1</sup>, J Morrow<sup>1</sup>, M Parton<sup>2</sup>, A Hiscock<sup>1</sup>, L Dewar<sup>2</sup>, S Brady<sup>3</sup>, D Hilton-Jones<sup>3</sup> and MG Hanna<sup>1,2</sup> <sup>1</sup>MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK; <sup>2</sup>Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK <sup>3</sup>Department of Clinical Neurology, University of Oxford, Level 6, West Wing, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK

Despite being the commonest myopathy acquired after the age of fifty, the natural history of Sporadic Inclusion Body Myositis (IBM) remains relatively unclear. There have been few cross-sectional studies of clinical features and fewer longitudinal studies of clinical progression in substantial IBM populations. Such data will be essential if adequately powered clinical trials are to be performed and clinic-pathological associations to be detected. We have established a dedicated IBM research clinic at the MRC Centre for Neuromuscular Diseases, in which patients undergo a full clinical assessment, physiotherapy assessment and quantitative myometry. Fifty patients have undergone protocol based assessment to date. With consent, DNA samples can be taken for whole exome analysis. Given the scattered nature of the IBM population, it will be crucial that additional centres caring for IBM patients are able to contribute easily to a centralised database. Therefore, we have devised a succinct proforma on which the key clinical and pathological information can be efficiently recorded. We have recently adapted this to facilitate transcription onto an electronic database called 'IBMnet' to which all hospitals with the relevant local ethical approval may contribute patient data. Oxford has recently joined this study. These initiatives have provided an excellent resource from which to recruit clinical trial participants and promise to advance our understanding of this disabling disease.

Acknowledgements: ARUK, MRC, MDC

#### **Poster 80**

##### **Benefits and adverse effects of glucocorticoids in boys with Duchenne Muscular Dystrophy: a UK perspective**

Ricotti V, Manzur AY, Scott E, Muntoni F, on behalf of NorthStar Clinical Network *The Dubowitz Neuromuscular Centre, Great Ormond Street Hospital and Institute of Child Health, University College, London, UK.*

Background: Glucocorticoids (GCs) slow decline in Duchenne Muscular Dystrophy(DMD). GCs are started in the early ambulant phase; and different regimes-associated profiles of efficacy and side effect exist. With the aim to optimize and standardise the care of ambulant DMD boys on GCs, the neuromuscular clinicians in the UK collect data in the NorthStar database.

Methods: Through the NorthStar database, clinical data was analysed for the period 2004 -2010. Ambulant DMD boys aged 4 -12 years attending 20 UK neuromuscular centres were recruited.

Results: Clinical longitudinal data was available for 305 boys: 113 on daily prednisolone (DP), 122 on intermittent prednisolone, 10-days-on/10days-off (IP). Moderate to severe side effects included: behavioural changes in 40% of DP and 26% of IP boys; weight gain 33% on DP and 29% on IP; height restriction 38%DP and 32%IP; Cushingoid features 28% DP and 11%IP; and vertebral fractures 6% and 3% respectively. 10% of boys discontinued steroids due to side effects (n=8), no

perceived benefit (n=7), and loss of ambulation (n=9). Longitudinal North Star Ambulatory Assessment (NSAA) data could be analysed for 73 boys on DP and 59 on IP. One-way ANOVA demonstrated group difference ( $p < 0.0001$ ) favouring DP: disability progression was delayed between 10-12 years of age.

Discussion: This audit demonstrates the importance of collecting information on clinical course and response to GCs in ambulant UK boys. The NorthStar network offers a unique opportunity to collect updated natural history data on a large number of DMD boys assessed and treated according to standardised protocols.

Acknowledgements: The support of Muscular Dystrophy Campaign for the NorthStar clinical Network (full list of participating centers [http://www.muscular dystrophy.org/assets/0001/5872/NSCN\\_collaborating\\_centres.pdf](http://www.muscular dystrophy.org/assets/0001/5872/NSCN_collaborating_centres.pdf)) is gratefully acknowledged.

### **Clinical trials supported by the MRC Centre**

Clinical trials linked to the MRC Centre and supported by different funding agencies including the Medical Research Council, Muscular Dystrophy Campaign, UK Department of Health, National Institutes of Health (USA), Food and Drug Administration (USA), AVI Biopharma and PTC Therapeutics, Alexion Pharmaceuticals, GlaxoSmithKline.

### **CTIMPs**

#### **Set-up Phase**

### **TAPP: THERAPEUTIC TRIAL OF POTASSIUM AND ACETAZOLAMIDE IN ANDERSEN-TAWIL SYNDROME**

**Status: Set-up Phase**

**Sponsor: University College London (UCL)**

**Funder: National Institutes of Health (NIH – USA)**

**PI: Prof Hanna**

**Recruitment target: 12**

Andersen-Tawil Syndrome (ATS) is a rare form of periodic paralysis that is associated with serious heart-rhythm abnormalities. ATS is characterized by a triad of episodic muscle weakness, long-QT syndrome with potentially fatal cardiac dysrhythmias and skeletal developmental anomalies. The underlying cause of this potentially fatal condition is only partly understood and there are no established treatments. Mutations in the KCNJ2 gene encoding Kir2.1, an inward-rectifying potassium channel account for approximately 60% of ATS cases (termed ATS1), the remaining 40% are presumed to have an as yet undetermined gene lesion and are designated ATS2. ATS1 and ATS2 are phenotypically indistinguishable.

The treatment of ATS has been largely anecdotal and empirical.

This proposal involves a multi-centre, placebo-controlled 'n of 1' study design of total duration 45 weeks. The expected total enrolment for this multi-centre study is 16 participants.

The aim of this study is to determine whether potassium supplements and/or acetazolamide alter the duration of muscle weakness and potentially life-threatening heart rhythm abnormalities in patients with ATS.

For information on the status of recruitment please contact Dr. James Burge at James.burge@uclh.nhs.uk or Gisela Barreto, Trials Coordinator at Gisela.barreto@uclh.nhs.uk.

### **DMD HEART PROTECTION TRIAL**

Full-Title: A double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy in genetically characterised males with DMD *without* echo-detectable left ventricular dysfunction.

**Status: Set-up phase**

**Sponsor: Newcastle NHS Foundation**

**Planned start date: 2011**

**Funder: British Heart Foundation**

**PI: Prof. Muntoni**

**Recruitment target: 140**

Duchenne muscular dystrophy [DMD] is an X-linked recessively inherited neuromuscular disorder due to a deficiency in the expression of the protein dystrophin on the inner aspect of cell sarcolemma. Its clinical course has traditionally been characterised by progressive weakness of proximal limb-girdle muscles and calf muscle hypertrophy.

Duchenne-affected individuals typically lose ambulation and become wheelchair-dependent before the age of 13 and die from cardio-respiratory failure at around the age of 20 years.

From the cardiology perspective, some 90% of males with DMD develop a severe, progressive form of cardiomyopathy. Twenty to 30% have evidence of left ventricular impairment on echocardiography by age 10 years.

Abnormalities in left ventricular function are evident in an even larger proportion of patients at all ages when more sensitive imaging techniques, such as tissue Doppler, magnetic resonance or metabolic imaging, are deployed. Despite the severity of cardiac involvement in DMD, cardiologists have largely ignored this particular inherited form of cardiomyopathy. This is due to the fact that, because of their inability to exercise, cardiac symptoms only occur terminally in DMD patients when all cardiac reserve has been eroded. Even today in most hospitals, cardio-active drug therapy is only started in patients with DMD when overt heart failure is evident and, even then, is typically deployed tentatively for symptom control, without any expectation that it can prolong life.

The objective of this trial is to determine whether the introduction of ACE inhibitor combined with beta-blocker therapy, before the onset of echo-detectable left ventricular dysfunction, can delay the age of onset and/or slow the rate of progression of cardiomyopathy compared to placebo in males with DMD.

This is a double-blind randomised, placebo-controlled Phase III trial of combined ACEinhibitor and beta-blocker therapy (perindopril and bisoprolol) over a minimum of three years and a maximum of five years. 140 participants (70 per arm) are to be enrolled and randomised.

For more information about the study please contact the trial coordinator on 020 7905 2639.

**Phase II, multicenter, randomized, adaptive, double-blind, placebo controlled Study to assess Safety and Efficacy of Olesoxime (TRO19622) in 3-25 year old Spinal Muscular Atrophy (SMA) patients**

**Status: Set-up phase**

**Sponsor: TROPHOS**

**Funder: Association Francaise contre les Myopathies**

**PIs: Francesco Muntoni, Hanns Lochmuller, Helen Roper**

**Recruitment target (UK): 30**

The UCL Institute of Child Health and Great Ormond Street Hospital for Children (London), Birmingham Heartlands Hospital, and Newcastle upon Tyne Hospitals Royal Victoria Infirmary have been invited to collaborate in this phase II clinical trial in non-ambulant patients with SMA II and III with a documented homozygous absence of SMN1 exon 7 and/or deletion and mutation on the other allele.

This is a multicentre, double-blind, randomized, placebo-controlled study in patients with SMA type 2 or non-ambulant type 3. The study will be conducted in multiple centres across Europe and will be sponsored by Trophos (a biopharmaceutical company based in France) and funded by AFM (Association française contre les myopathies). The aim is to assess efficacy, futility, safety and tolerability of a new drug called olesoxime. This is a neuroprotective drug that acts by interacting with protein components of the mitochondrial permeability transition pore (mPTP), preventing the release of apoptotic factors and in turn neuronal death. Olesoxime has displayed an excellent safety profile and has been well tolerated in phase I clinical trials in healthy subjects.

For each participant, this phase II study will involve a 4 week screening period followed by a 24 month (104 week) treatment period. Following screening procedures and confirmation of eligibility, subjects will be randomised to receive either olesoxime or placebo in a 2:1 ratio. Olesoxime (or matched placebo) will be taken daily with evening meal as a liquid formulation at a dose of 10mg/kg. 150 subjects in total will be recruited, with a target of 30 patients in the UK. Recruitment is planned to be completed in 6 months. It is possible a dose adjustment may be made once 45



patients across Europe have been received study drug for 3 months based on a review by a designated independent Data Monitoring Committee.

The patients to be recruited should be at least 3 years of age but younger than 26 years at the time of enrolment, with the age of onset of symptoms to be at 3 years of age or younger. They should not be taking any medication intended for the treatment of SMA within 30 days prior to being enrolled on the study. Eligible patients can be taking oral salbutamol as long as this has been commenced at least six months prior to enrolment on the study and remains at a stable dose during the study period. Participation in another investigational drug or therapy study within 3 months of enrolment is an exclusion criterion, as well as a hypersensitivity to sesame oil and use of medications that could interfere with olesoxime absorption (including cholesteramine, fibrates, fish-oils, niacin, phytosterols and ezetimibe).

Further information about this study can be obtained from the Clinical Trials Coordinator on 020 7905 2639.

### **Open Trials**

#### **HYP HOP: DICHLORPHENAMIDE vs. PLACEBO FOR PERIODIC PARALYSIS**

Full Title: Double-blind, placebo-controlled, parallel group, phase III study comparing dichlorphenamide vs. placebo for the treatment of periodic paralysis

**Status: Open to Recruitment**

**Sponsor: University Rochester**

**Funder: National Institutes of Health (NIH - USA)**

**PI: Prof. Hanna**

**Patients recruited: 10; target 40**

This is a phase III trial into Periodic Paralysis. This proposal involves a multi-centre, double-blind, placebo-controlled parallel group, nine-week studies comparing the effects of dichlorphenamide(DCP) vs placebo in patients with period paralysis (Hyper, Hypokalemic periodic paralysis). The 9-week studies will investigate the prevention of attacks of weakness and it will be followed by 1-year extensions without placebo to compare the long term effects of DCP on the course of the diseases and on inter-attack weakness. Approximately 40 participants will be recruited from the United Kingdom.

For information on the status of recruitment please contact Dr. James Burge at [James.burge@uclh.nhs.uk](mailto:James.burge@uclh.nhs.uk) or Gisela Barreto, Trials Coordinator at [Gisela.barreto@uclh.nhs.uk](mailto:Gisela.barreto@uclh.nhs.uk).

#### **THERAPEUTIC TRIAL OF MEXILETINE IN NON-DYSTROPHIC MYOTONIA**

Full Title: A Phase II Randomised, Double-Blind, Placebo controlled, Cross-Over Study to Investigate the Efficacy of Mexiletine in Patients with Non-Dystrophic Myotonia

**Status: Open to Recruitment**

**Sponsor: University College London (UCL)**

**Funder: Food and Drug Administration (FDA – USA)**

**PI: Prof. Hanna**

**Patients recruited: 15; target 15**

The non-dystrophic myotonia (NDM) is a group of rare neuromuscular disorders that causes episodes of muscle stiffness (known as myotonias) and paralysis. Predominantly the muscles of the face, hands and legs are affected. In addition to these episodes a permanent and debilitating muscle weakness can develop. The optimal treatment for these disorders is unknown.

Non-dystrophic myotonias are due to abnormalities of ion channels present in skeletal muscle membranes. There is experimental evidence that drugs like mexiletine which block the abnormal function of these ion channels allow the muscle to perform normally.

The study aims to test the efficacy of mexiletine in the treatment of the non-dystrophic myotonias.

This proposal involves a multi-centre, double-blind, placebo-controlled cross over trial of total duration nine weeks.

Approximately fifteen participants will be enrolled in the UK at the National Hospital for Neurology and Neurosurgery.

For information on the status of recruitment please contact Dr. Dipa Raja Rayan at [d.rajarayan@ion.ucl.ac.uk](mailto:d.rajarayan@ion.ucl.ac.uk) or Gisela Barreto, Trials Coordinator at [Gisela.barreto@uclh.nhs.uk](mailto:Gisela.barreto@uclh.nhs.uk)

### **ARIMOCLOMOL FOR SPORADIC INCLUSION BODY MYOSITIS (IBM)**

Full Title: A Randomised, Double-blinded, Placebo-controlled Pilot Study Assessing the Safety and Tolerability of Arimoclomol in Adult Patients with Sporadic Inclusion Body Myositis

**Status: Open to Recruitment**

**Sponsor: University College London (UCL)**

**Funder: Medical Research Council (MRC)**

**PI: Prof. Hanna**

**Patients recruited: 11; target 12**

Sporadic Inclusion Body Myositis (IBM) is the commonest acquired disease of muscle affecting people aged 50 years and over. This is a progressive and debilitating disease with both muscle weakness and wasting, characteristically of the quadriceps and finger flexors. Over time the condition can lead to severe disability, falls and swallowing impairment. Affected muscle tissue demonstrates inflammation and degeneration.

Arimoclomol is a new compound which acts by enhancing a normal, inbuilt protective cell reaction to stresses. The products of this response are 'Heat Shock Proteins (HSPs) which counteract processes that end up leading to abnormal protein deposition and to damage mediated by inflammation.

This proposal involves a multi-centre, double-blind, placebo-controlled parallel study of total duration twelve weeks.

This study proposal aims to assess the safety and tolerability of Arimoclomol (100 mg TDS) as compared with placebo over 4 months of treatment in patients with IBM.

Recruitment will take place at the National Hospital for Neurology and Neurosurgery and twelve patients will be enrolled.

For information on the status of recruitment please contact Dr. Pedro Machado at [p.machado@ion.ucl.ac.uk](mailto:p.machado@ion.ucl.ac.uk) or Gisela Barreto, Trials Coordinator at [Gisela.barreto@uclh.nhs.uk](mailto:Gisela.barreto@uclh.nhs.uk).

### **GSK/Prosensa clinical trial in DMD boys with study drug GSK2402968 (PRO051)**

Full Title: A phase II, double-blind, exploratory, parallel-group, placebo-controlled clinical study to assess two dosing regimens of GSK2402968 for efficacy, safety, tolerability and pharmacokinetics in ambulant subjects with Duchenne muscular dystrophy

**Status: Ongoing**

**Sponsor: GlaxoSmithKline**

**Funder: GlaxoSmithKline**

**PIs: Volker Straub, Francesco Muntoni**

**Patients recruited: 8; target (UK) 8**

A multicentre trial with this study drug is recruiting DMD boys in UK at the Great Ormond Street Hospital(GOSH), London and at the Royal Victoria Infirmary, Newcastle. It is a Phase IIa, double blind, exploratory, parallel clinical trial to assess the optimal dose of GSK2402968 for safety, tolerability and efficacy, in ambulant patients with DMD.

This study is designed to explore efficacy and safety of GSK2402968 given as a continuous regimen and an intermittent regimen over 24 and 48 weeks.

#### Objective(s)

Primary objective:

- To assess the efficacy of 2 different dosing regimens of subcutaneous GSK2402968 administered over 24 weeks in ambulant subjects with DMD.

Secondary objectives:

- To assess the safety and tolerability of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.
- To assess the PK of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.
- To assess long term efficacy of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.

#### Study Design

The study aims to randomise 54 subjects. There will be 2 parallel cohorts. Each cohort will include 16 subjects on GSK2402968 and 8 subjects on matched placebo (2:1 ratio).

Further information about this study can be obtained from the Clinical Trials Coordinator on 020 7905 2639.

### **Investigation of the ability of Otelixizumab to inhibit in vitro antigen-specific T cell responses from Myasthenia Gravis patients**

**Status: Open to Recruitment**

**Sponsor/Funder: GlaxoSmithKline**

**PI: Prof Kullmann**

**Patients recruited: 11; target 40**

Myasthenia Gravis (MG) is the best understood autoimmune disease (a disease in which the immune system attacks some part of the body). This attack is directed by various parts of the immune system.

There is a continued search for newer drugs that will be of benefit in the treatment of MG. There is a continued search for newer drugs that will be of benefit in the treatment of MG. Otelixizumab has been identified as a possible treatment for MG. However before clinical trials can be considered additional information is needed to determine how it interacts with the immune system of patients with MG.

In this study adult patients with MG will be invited to provide blood samples (50 ml) for research purposes. Blood collected from patients will be used for Tcell assay and autoantibody assay development. Patients may be asked to provide a repeat blood sample (additional 50ml) after 46 months following the initial collection to see if T cell activation changes over time. Up to 40 participants will be enrolled in the UK.

The study is being sponsored by GlaxoSmithKline group of companies.

For information on recruitment contact Natalie James (Natalie.James@uclh.nhs.uk).

### **THERAPEUTIC TRIAL OF LITHIUM CARBONATE IN MND/ALS (LiCALS)**

**Full title: A double-blind, randomised, placebo controlled trial of lithium carbonate in patients with amyotrophic lateral sclerosis.**

**Status: Ongoing (closed to recruitment)**

**Sponsor: University College London Hospitals NHS Foundation Trust**

**Start date: June 2009**

**Funder: Motor Neurone Disease Association, and NIHR  
UCL PI: Dr Richard Orrell**

Recent research suggested that lithium carbonate may be effective in lowering the progression of MND/ALS. Lithium may protect motor neurons through a range of mechanisms, including improving the transport of proteins along the motor neuron, improving the transport of mitochondria, and activating cell survival factors. In one study, lithium prolonged survival in a mouse model of MND/ALS.

This is a multi-centre UK study, involving 215 patients with MND/ALS, taking lithium or placebo, for 18 months. The trial is designed to assess the safety, efficacy and tolerability of lithium in combination with riluzole as a treatment for MND/ALS. Assessments include survival, symptoms, quality of life, and function. Participants are randomised to take lithium or placebo, the level of lithium in the blood is monitored, and the dose of lithium (and placebo) adjusted as needed.

#### **GSK1223249 in MND/ALS (the Nogo-A study)**

**Full title: A Phase I, multi-center, randomized, placebo-controlled, double-blind, single and repeat dose escalation of a drug to treat ALS.**

**Status: Recruiting**

**Sponsor: Royal Free Hampstead NHS Trust**

**Start date: September 2010**

**Funder: GlaxoSmithKline**

**UCL PI: Dr Richard Orrell**

GSK 1223249 is a new drug developed by GlaxoSmithKline, that targets a protein called Neurite Outgrowth Inhibitor (Nogo-A), which impairs neurone regeneration. There is evidence of increased Nogo-A, which impairs neuron regeneration, in muscle of people with MND/ALS. By blocking the effect of Nogo-A, GSK1223249 may be an effective treatment for the disease. GSK1223249 delays symptom onset and prolongs survival in a mouse model of MND/ALS.

The trial will provide safety and tolerability information, together with biomarker and functional information. This may lead to further trials to assess effectiveness. The study includes an infusion of the drug (or placebo), with a muscle biopsy taken before and following the infusion, together with other monitoring assessments.

For further information please contact Dr Richard Orrell ([r.orrell@ucl.ac.uk](mailto:r.orrell@ucl.ac.uk))

#### **BIOMARKER STUDIES IN MND/ALS**

**Full title: Characterisation of a panel of disease biomarkers in peripheral blood from individuals with motor neuron disease**

**Sponsor: University College London Hospitals NHS Foundation Trust**

**Start date: May 2009**

**Funder: Motor Neurone Disease Association**

**UCL PI: Dr Richard Orrell**

Motor neuron disease (MND) is an adult-onset neurodegenerative disease and one of the commonest neuromuscular disorders. The speed of progression of MND varies among individuals and the condition can develop with different clinical manifestations. Currently, there are no blood tests that could help us to predict the speed of progression of the disease and the likely clinical manifestations (e.g. predominant involvement of speech and swallowing or of the limb muscles). We are testing specific disease biomarkers in the blood. To assess change over time, a blood sample is taken every 3 months. The sample has to be carefully processed as soon as it is taken to preserve the quality of the blood contents. We are studying a range of blood constituents including proteins, DNA and RNA. From some participants we also collect samples of cerebrospinal fluid. If repeated samples are not possible, a single sample of blood for DNA studies is also helpful. We also

examine samples from participants without MND/ALS, and individuals with similar but unrelated neuromuscular conditions. Parallel studies of biomarkers in an animal model of ALS are informing our choice of biomarkers. The study is in collaboration with Queen Mary University of London, and other participating centres.

### **Closed Trials**

#### **RANDOMISED DOUBLE-BLIND PLACEBO CONTROLLED TRIAL OF LONG-TERM ASCORBIC ACID TREATMENT IN CHARCOT-MARIE-TOOTH DISEASE TYPE 1A**

**Status: Completed.**

**Sponsor: University College London**

**Funder: Muscular Dystrophy Campaign (MDC)**

**PI: Prof. Reilly**

**Patients recruited: 50**

Charcot-Marie-Tooth disease 1A (CMT1A) is associated with a duplication of the peripheral myelin protein 22 (PMP22) gene. To date there is no pharmacological treatment for CMT1A patients. Treatments and therapy for CMT is restricted to symptomatic treatments such as physiotherapy and surgery for skeletal deformities.

Recently, treatment with ascorbic acid (AA) has been shown to be effective for transgenic mice over-expressing PMP22, a model of the human disease. Treated animals had much less severe neuropathy as compared to untreated controls as shown by clinical and histological findings. Some clinical parameters even improved during treatment.

This is a phase III prospective, multi-centre, randomised, double-blind, placebo-controlled study aiming to evaluate the efficacy of AA treatment in CMT1A.

The study is now complete. Fifty participants were enrolled in the UK site at the National Hospital for Neurology and Neurosurgery.

#### **A PHASE IIb EFFICACY AND SAFETY STUDY OF PTC124 IN SUBJECTS WITH NONSENSE MUTATION-MEDIATED DUCHENNE AND BECKER MUSCULAR DYSTROPHY**

**Status: Completed**

**Sponsor: PTC Therapeutics**

**Funder: PTC Therapeutics**

**PIs: Prof. Muntoni, Prof. Bushby**

**Patients recruited: 11**

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder affecting young boys. The condition is disabling and life-threatening. A small subset of boys are classified as having Becker muscular dystrophy (BMD), a phenotypically milder form of the dystrophic muscle disease.

In approximately 10 to 15% of boys with DMD and BMD the causative defect is the presence of a nonsense mutation in the dystrophin gene that truncates dystrophin protein production by introducing a premature stop codon into the dystrophin messenger ribonucleic acid (mRNA).

PTC124 is a novel, orally bioavailable, small-molecule drug that promotes ribosomal read-through of mRNA containing a premature stop codon. Through this mechanism of action, PTC124 has the potential to overcome the genetic defect in boys for whom a nonsense mutation causes DMD/BMD.

In vitro studies in cell lines with dystrophin nonsense mutations have shown that PTC124 can restore production of the missing dystrophin gene.

This is an international, multi-centre, randomised, double-blind, placebo-controlled, dose-ranging, efficacy and safety study.

The study primary aim is to evaluate the effect of PTC124 on ambulation as assessed by the distance walked during a 6-minute walk test (6MWT).

The double-blind arm of the study randomised 174 participants worldwide which are to be followed for a period of 12 months. At the completion of the blinded treatment, all compliant participants were eligible to receive open-label PTC124 in a separate extension study.

(Ataluren is now the non-proprietary generic name for PTC124).

This work has been completed.

The preliminary findings from the Ataluren Study 007 did not show significant muscle improvement in the patients who participated in the study. The study was therefore discontinued. An update on this study was presented at the International Congress on Neuromuscular Diseases, Naples, Italy, 17-22 July 2010 by Professor Kate Bushby. Details of this presentation is available on [www.ptcbio.com](http://www.ptcbio.com) Briefly, analysis showed that, on average, patients treated with low-dose ataluren experienced better outcomes on measures of efficacy than patients treated with high-dose ataluren or placebo - this phenomenon is not unique for ataluren and has been observed with other drugs for other diseases. Further analysis of efficacy data is ongoing.

### **ANTISENSE OLIGONUCLEOTIDE INDUCED EXON SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY**

This initiative is led by the MDEX consortium (The MDEX consortium led by Professor Muntoni, is a multidisciplinary enterprise to promote translational research into muscular dystrophies, and is formed by the clinical groups of Professor Francesco Muntoni (UCL Institute of Child Health) and Professor Kate Bushby and Professor Volker Straub (Newcastle University), and scientists from Imperial College London (Professor Dominic Wells), UCL Institute of Child Health (Dr Jennifer Morgan), Royal Holloway University of London (Professor George Dickson and Dr Ian Graham), Oxford University (Dr Matthew Wood) and University of Western Australia (Prof Steve Wilton). In addition, the charities Muscular Dystrophy Campaign (MDC), Action Duchenne and Duchenne Family Support Group also participate in the Consortium, [www.mdex.org.uk](http://www.mdex.org.uk)).

The current two trials led by the consortium are mentioned below.

### **RESTORING DYSTROPHIN EXPRESSION IN DUCHENNE MUSCULAR DYSTROPHY: A PHASE I/II CLINICAL TRIAL USING AVI-4658**

**Status: completed**

**Sponsor: Imperial College London**

**Funder: Department of Health (DoH)**

**PI: Prof. Muntoni**

**Patients recruited: 8**

The primary scope of the trial is to assess efficacy (dystrophin production) and safety of intramuscular administered morpholino oligomer directed against exon 51 (AVI – 4658 PMO).

Antisense therapy with the use of antisense oligomers has the potential to restore effectively the production of dystrophin, the defective protein, in >70% of DMD. This could result in increased life expectancy through improved muscle survival and function. Recent scientific research has demonstrated the potential of this technique to skip mutated dystrophin exons, restore the reading frame and generate functional dystrophin protein. Having demonstrated proof-of-principle in human cell culture and animal model studies, we now intend to determine efficacy and safety of this approach to induce dystrophin exon skipping in children with DMD. This study is aimed at children with Duchenne muscular dystrophy above the age of 10 years with mutations than can be rescued by the skipping of exon 51 [45-50; 47-50; 48-50; 49-50; 50; 52; 52-63].

This work has been completed and outcome data published in the journal Lancet Neurology (Volume 8, Issue 10, Pages 918 - 928, October 2009)

## **DOSE-RANGING STUDY OF AVI-4658 TO INDUCE DYSTROPHIN EXPRESSION IN SELECTED DUCHENNE MUSCULAR DYSTROPHY (DMD) PATIENTS – (Systemic study)**

**Status: Closed to recruitment**

**Sponsor: AVI Biopharma**

**Funder: Medical Research Council (MRC) and AVI Biopharma**

**PI: Prof. Muntoni**

**Patients recruited: 19**

This is a safety study of AVI-4658 (a 30-base phosphorodiamidate Morpholino oligomer [PMO]), to skip exon 51 of the dystrophin gene in relevant subjects with DMD.

This is an open-label, two-centre, dose-ranging comparative clinical study of duration twelve weeks.

The objectives of the study are to assess safety and to select the optimum dose that elicits at least 10% de novo dystrophin-positive fibres and dystrophin in a sentinel muscle group after an intravenous AVI-4658 dosing regimen.

A total of up to 16 subjects (ambulatory paediatric males, aged  $\geq 5$  and  $\leq 15$  years of age) will be enrolled in this study, consisting of four treatment cohorts and four subjects per cohort. It is expected that there will be four treatment arms ranging from 0.5 mg/kg to 4 mg/kg. All subjects will receive 12 weekly intravenous infusions of AVI-4658.

Precedent studies have demonstrated that AVI-4658 might have therapeutic relevance in managing DMD for boys whose frame-shifted dystrophin gene lesion could be restored after excision of exon 51 if sufficient drug is translocated into the nucleus of the afflicted muscle cell.

This trial was conducted in London and Newcastle.

A total of 19 subjects (12 at GOSH and 7 at RVI, Newcastle) were recruited and final data is being analysed for submission to regulatory authorities in Europe and the USA. Outcome data were presented at the World Muscle Society, 12-16 October 2010 in Japan and a manuscript has been submitted.

## **ECULIZUMAB FOR MYASTHENIA GRAVIS**

Full Title: A Randomised, Double-Blind, Placebo-controlled, Cross-over, Multicenter Study of Eculizumab in Patients with Generalised Myasthenia Gravis (GMG) who have Moderate to Severe Muscle Weakness Despite Treatment with Immunosuppressants

**Status: Closed**

**Sponsor/Funder: Alexion Pharmaceuticals, Inc.**

**PI: Prof. Dimitri Kullmann**

This is a randomized, double-blind, placebo-controlled, cross-over, multicenter study to evaluate the safety and efficacy of eculizumab for the treatment of patients with myasthenia gravis. Myasthenia gravis (MG) is an acquired autoimmune syndrome caused by the failure of neuromuscular transmission, which results from the binding of autoantibodies to proteins involved in signaling at the neuromuscular junction (NMJ). These proteins include the nicotinic AChR or, less frequently, a muscle-specific tyrosine kinase (MuSK) involved in AChR clustering.

Current available treatments for myasthenia gravis aim to modulate neuromuscular transmission, to inhibit the production or effects of pathogenic antibodies, or to inhibit inflammatory cytokines. There is currently no specific treatment that corrects the autoimmune defect in MG.

Eculizumab is a humanized murine monoclonal antibody that blocks the activation of complement by selectively binding to C5 and preventing the enzymatic cleavage of C5 to C5a and C5b. The blockade of complement activation at this point in the cascade has been shown to prevent the proinflammatory effects of both C5a and C5b, especially the chemotaxis of inflammatory cells, and MAC (C5b-9)-mediated cell activation and lysis. Since eculizumab effectively inhibits complement,

especially MAC formation, it is a potentially effective therapeutic approach for diseases such as MG in which the formation of the MAC and/or the release of C5a leads to localized destruction of the postsynaptic NMJ membrane and play an important role in the disease process.

Patients will receive approximately 22 infusions including 11 infusions of eculizumab and 11 infusions of placebo. The estimated duration of a patient's participation is approximately 41 weeks.

For more information about the study please contact Dr. Jennifer Spillane at [jspillane@ion.ucl.ac.uk](mailto:jspillane@ion.ucl.ac.uk) or Natalie James at [Natalie.James@uclh.nhs.uk](mailto:Natalie.James@uclh.nhs.uk).

### **Natural History – Longitudinal Studies**

#### **Set-up Phase**

#### **OUTCOME MEASURES IN SMA TYPE II AND III**

**Status: Recruitment to commence shortly**

**Funder: SMA Europe**

**PI: Prof Muntoni**

This project provides an excellent opportunity as for the first time, ten leading neuromuscular centers in Europe which have been involved in the development and validation of functional scales for SMA will collaborate to validate and cross validate measures that have been suggested to be the most suitable for multicentric trials by a large international consensus, but have not been tested in large multicentric studies yet.

One hundred and thirty patients across Europe affected by type II and type III SMA will be enrolled and assessed at baseline and 6 and 12 months later. Non ambulant patients will be assessed using the modified version of the Hammersmith Motor Functional Scale while ambulant patients will be assessed using the extended module of the Hammersmith Motor Functional Scale and timed items, the 6 minute walk and a step activity monitor. All patients will also be assessed using the MFM, that covers the whole range of activities for both ambulant and non ambulant patients. All measures will undergo a process of validation including inter observer reliability. This information will be most valuable for any future trial and will make the groups involved ready to participate to future collaborative studies saving a lot of time on the preliminary aspects (validation, reliability, training) that will be fulfilled by the present study. The study will also provide natural history data for a 12 month period on patients with SMA II and III.

Further information can be obtained from the Trials Coordinator or Research Physiotherapist on 020 7905 2639.

#### **Open Trials**

#### **ANDERSEN-TAWIL SYNDROME: GENOTYPE AND PHENOTYPE CORRELATION AND LONGITUDINAL STUDY**

**Status: Open to recruitment**

**Sponsor: University College London**

**Funder: National Institutes of Health (NIH – USA)**

**PI: Prof. Hanna**

**Patients recruited: 11 Target 12**

Andersen-Tawil syndrome is a neuromuscular disorder caused by a mutation in the KCNJ2 gene which codes for the inwardly rectifying potassium channel Kir2.1. A number of different mutations in this gene have already been identified in affected individuals. This disorder is characterised by the triad of periodic paralysis, developmental abnormalities and cardiac arrhythmias.

This project is a natural history trial into Andersen-Tawil Syndrome. The aim of the trial is to study the relationship between the genetic abnormalities underlying the disorder and the diverse clinical features.



Eleven patients have been enrolled so far at the National Hospital for Neurology and Neurosurgery.

For information on the status of recruitment please contact Dr. Dipa Raja Rayan at [d.rajarayan@ion.ucl.ac.uk](mailto:d.rajarayan@ion.ucl.ac.uk).

### **EPISODIC ATAXIA SYNDROME: GENOTYPE-PHENOTYPE CORRELATION AND LONGITUDINAL STUDY**

**Status: Recruiting**

**Sponsor: University College London**

**Funder: National Institutes of Health (NIH – USA)**

**PI: Prof. Hanna**

**Patients recruited: 36 target 36**

Episodic Ataxia Syndrome is a rare, genetic disease that causes recurrent episodes of dizziness and incoordination.

The majority of cases are likely caused by an inherent genetic mutation. However in some patients the mutation is unidentifiable. The purpose of this study is to collect prospective standardized data from subjects to better define the clinical phenotype of the EAs and to establish clinically relevant endpoints for use in therapeutic trials.

The study will also :

- Fully characterize the clinical spectra and the natural history of genetically defined EA.
- Systematically investigate phenotypic differences between EA subjects harboring KCNA1/CACNA1A mutations and those that do not.

This proposal involves a multi-center cross-sectional data collection analysis as well as a prospective longitudinal study. Since EA is a chronic disease whose course is measured in years rather than months, the subjects will be followed longitudinally at a yearly interval for a period of two years.

For information about the study please contact Tracey Graves at [tracey.graves@btinternet.com](mailto:tracey.graves@btinternet.com).

### **CMT: A NATURAL HISTORY STUDY**

Full Title: Charcot-Marie-Tooth Disease and related disorders: A Natural History Study

**Status: Open to Recruitment**

**Sponsor: University College London Hospitals**

**Funder: National Institutes of Health (NIH – USA)**

**PI: Dr Reilly/Prof Muntoni**

**Patients recruited: 72; target (UK) >50**

Charcot-Marie-Tooth Disease (CMT) and related disorders (distal hereditary motor neuropathy (dHMN) and hereditary sensory and autonomic neuropathy (HSAN)) are a clinically and genetically heterogenous group of disorders affecting approximately 1 in 2500 people.

People with this condition present with upper and lower limb weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles. Despite the clinical similarities among patients with CMT the group is genetically heterogeneous. Advances have been made in identifying the genes that cause CMT and the molecular organisation of the peripheral nervous system (PNS) nevertheless the optimal management and treatment of the different variants of this disorder is not known and moreover natural history data is lacking for most forms of inherited neuropathies.

This is a 5 year study that will be conducted by four centres in United States and two centres in the UK (National Hospital for Neurology and Neurosurgery and Great Ormond Street Hospital). The aim of the project is to fully characterise the features of different types of CMT and the longitudinal progression of the disease. The data will also be used to establish clinical relevant endpoints for use

in therapeutic trials. The identification and genetic characterisation of patients will facilitate the recruitment of participants for future therapeutic trials. Ultimately the information gained with this study will lead to the improvement in the treatment and management of CMT.

The study is also seeking to establish an appropriate paediatric impairment scoring method for CMT and establish a database for the inherited neuropathies. The study will include both adult and paediatric patients. Evaluations will consist of a neurological history and examination, nerve conduction velocity (NCV) study and in some selected cases skin biopsy.

This is a NIH funded study. At least fifty patients will be enrolled at the National Hospital for Neurology and Great Ormond Street Hospital.

For more information about the study please contact Dr. Matilde Laura at [m.laura@ion.ucl.ac.uk](mailto:m.laura@ion.ucl.ac.uk).

## **MITOCHONDRIAL DISEASE COHORT**

**Status: Open to Recruitment**

**Sponsor: The University of Newcastle Upon Tyne**

**Funder: MRC**

**PI: Dr R McFarland**

**patients recruited: 468; target 1500**

The current project proposes to develop a cohort of UK patients with mitochondrial diseases. The details are to be stored in a database that will enable clinicians to gain adequate information for future clinical trials.

Mitochondrial diseases present a huge challenge to patients and doctors because no effective treatment is available. The extremely diverse phenotypic presentation of mitochondrial disease has previously limited cohort development.

The cohort will comprise symptomatic adults and children, in whom a mitochondrial disease phenotype and (where possible) genotype, have been confirmed. Asymptomatic individuals who have requested genotyping and proved positive will also be included. Genotyping is important because the same mitochondrial phenotype may be caused by several distinct mutations in either the mitochondrial or nuclear genomes. Phenotype will be characterized in all individuals (symptomatic and asymptomatic) on the basis of clinical history, clinical examination and detailed investigation.

Two centres will receive referrals (Newcastle University and University College London Hospitals). The database will physically be stored at Newcastle University and it will have a dedicated, electronic secure server.

The project anticipates collecting details on 1500 patients in total.

For information on the status of recruitment please contact Dr. Robert Pitceathly (London) [r.pitceathly@ion.ucl.ac.uk](mailto:r.pitceathly@ion.ucl.ac.uk) or Geoff Bell (Newcastle) [geoff.bell@nuth.nhs.uk](mailto:geoff.bell@nuth.nhs.uk).

## **THE NATURAL HISTORY OF INCLUSION BODY MYOSITIS (IBM Net)**

**Status: Open to Recruitment**

**Sponsor: University College Hospitals**

**Funder: MDC**

**PI: Dr Matt Parton**

**Patients recruited: 39; target 64**

Inclusion body myositis (IBM) is probably the commonest muscle disease beginning in those aged over 50. It leads to progressive disability with, classically, a characteristic pattern of muscle

involvement. However it is poorly understood: its cause is unknown, there is no conclusive diagnostic test and it has no treatment. Furthermore, information on the pattern and prognosis of IBM is more based on anecdote from clinical experience, rather than firm fact. The largest published series of data on the natural history of the illness followed only eleven patients for six months.

The current project seeks to better characterise IBM by gathering clinical data from as many cases as possible.

Serial standardised assessment (annually for five years) will chart disease progression and so both expand and strengthen knowledge of the natural history of the illness. Furthermore, establishment of a cohort of reliably-defined cases will build a valuable resource that could potentially form the starting-point for future studies.

For information on the status of recruitment please contact Dr. Pedro Machado at [p.machado@ion.ucl.ac.uk](mailto:p.machado@ion.ucl.ac.uk)

## **PERIPHERAL NEUROPATHY OUTCOME MEASURES STANDARDISATION STUDY (PERINOMS)**

**Status: Open**

**Sponsor: Erasmus Medical Center**

**PI: Dr M Lunn**

**Patients recruited: 110 (NHNN 4); overall target 120**

The current study aims to expand the clinimetric knowledge on outcome measures at various levels of outcome (pathology, impairment, activity & participation limitation, and quality of life) in autoimmune polyneuropathies, particularly in GBS, CIDP, MMN, MGUSP, and autoimmune small fibre neuropathies (AI-SFN). Also, the general applicability of an autonomic symptoms scale plus some selected activity limitation scales will be examined.

Outcome measures will be assessed in a cross-sectional and longitudinal group of patients at the level of:

- Pathology: Intraepidermal nerve fibre (IENF) density will be assessed in patients with GBS, CIDP, MGUSP, and AI-SFN (in sarcoidosis). IENF density will be examined regarding its correlation with other outcome measures (validity), its reliability (intra-observer and inter-observer), and its responsiveness to clinical changes over time.

- Impairment: comparison studies, evaluating the validity, reliability, and responsiveness will be performed between MRC sumscore versus NIS motor subset, INCAT sensory sumscore versus NIS sensory sumscore, and hand-held Vigorimeter versus Jamar dynamometer. Also, the correlation of electrophysiological studies with other impairment outcome measures will be evaluated. Finally, the scientific soundness of the modified Dutch composite autonomic symptoms scale (mdCompass) will be examined.

- Activity limitation: comparison studies, evaluating the validity, reliability, and responsiveness will be performed between the ODSS and an overall neuropathy limitations scale (ONLS). Also, a newly devised weighted (based on Rasch analyses) activity and participation scale will be constructed, aiming specifically on the limitations in patients with polyneuropathy.

- Quality of life: Disease-specific versus generic quality of life measures will be assessed, determining their clinimetric soundness and by comparison studies in the various polyneuropathy groups.

The ultimate goal of the current study will be the presentation of a specific minimum core set of outcome measures to be used in future clinical and follow-up studies in patients with polyneuropathy, mainly those patients with autoimmune mediated polyneuropathies. The study will be performed in collaboration with several local, European, and USA neurological centres with great experience in dealing with inflammatory neurological disorders.

## **Closed Trials**

### **NON-DYSTROPHIC MYOTONIAS: GENOTYPE AND PHENOTYPE CORRELATION AND LONGITUDINAL STUDIES**

**Status: Closed to recruitment**

**Sponsor: University College London**

**Funder: National Institutes of Health (NIH – USA)**

**PI: Prof. Hanna**

**Patients recruited: 20 target 20**

This multi-centre project involves a prospective, cross-sectional and longitudinal natural history in non-dystrophic myotonias (NDM).

The aim is to collect standardized data from NDM patients, to include clinical symptoms, exam findings, as well as the results of strength, functional, and electrophysiological testing. Genetic testing will permit precise identification of individual NDM subtype. This information will allow for the identification and implementation of appropriate endpoints in studies of potential treatments.

This is a NIH funded study. Twenty patients were enrolled at the National Hospital for Neurology and Neurosurgery.

For more information about the study please contact Dr. Dipa Raja Rayan at [d.rajarayan@ion.ucl.ac.uk](mailto:d.rajarayan@ion.ucl.ac.uk).

## **Exercise Studies**

### **Set-up Phase**

None

## **Open Trials**

### **EXERCISE TRAINING IN PATIENTS WITH MITOCHONDRIAL DISEASE: ASSESSING THE BENEFITS**

**Status: Recruiting**

**Sponsor: University Newcastle**

**Funder: Muscular Dystrophy Campaign (MDC)**

**PI: Prof Turnbull**

**Patients recruited: 6(5NCL 1UCL) Target 6**

Mitochondrial myopathies are a very important group of muscle diseases associated with weakness, pain and fatigue. At present, treatment options are very limited.

Exercise therapy has been found to have some benefit in this group of patients and we wish to explore this further in terms of both strength and endurance.

The aim of this study is to demonstrate that strength exercise training is an effective approach to therapy in certain patients with mitochondrial myopathy, specifically those with sporadic mutations in mitochondrial DNA. Based on our previous research studies, we believe that such training will improve muscle strength, mitochondrial function, exercise tolerance and overall quality of life.

The main objectives will be:

To confirm that endurance training in patients with mitochondrial abnormalities improves quality of life, exercise tolerance and oxidative capacity.

To determine the ability of resistance muscle strength training to improve skeletal muscle strength and oxidative capacity by incorporation of satellite cells into mature myofibres.

Participants are expected to commit to an exercise training and testing over a period of 4 to 8 months.

The study will include patients between the ages of 18 and 65 years who have had a previous muscle biopsy showing a defect in skeletal muscle mitochondrial DNA that is either in the form of a sporadic point mutation or single large-scale deletion. Patients who have this type of mutation and do not have any family members that are affected and have no major cardiac involvement, hypertension, pulmonary or peripheral vascular disease that may complicate findings.

For information about recruitment contact Geoff Bell at [geoff.bell@nuth.nhs.uk](mailto:geoff.bell@nuth.nhs.uk) or Dr Robert Pitceathly at [r.pitceathly@ucl.ac.uk](mailto:r.pitceathly@ucl.ac.uk).

## **CARDIAC ADAPTATIONS TO EXERCISE IN MITOCHONDRIAL DISEASE**

**Status: Recruiting**

**Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust**

**Funder: MRC**

**PI: Prof D M Turnbull/Dr MI Trenell,**

**Patients recruited: 9; target 24**

Twenty four people with mitochondrial disease will take part in the study. Participants will undergo cardiac, cognitive and movement examination and then they will be randomised into two groups. They will receive either; exercise counselling and support (n = 12) or continue standard care (n = 12) over a 16 week period. At the end of the 16 week period baseline measures will be repeated. Participants to be studied will have biopsy proven mitochondrial disease (age 18–60 years; BMI 20–35 kg/m<sup>2</sup>; and do not take part in regular exercise). Subjects with heart disease that would produce an adverse response to exercise will be excluded. Subjects with significant kidney disease or in vivo ferrous material will be excluded also as these are contra-indications to the use of gadolinium-based contrast agents and magnetic resonance imaging respectively. Magnetic resonance and echocardiographic evaluation of cardiac function as well as movement and cognitive function will be assessed at baseline and at 16 weeks. A progressive exercise test will be undertaken at baseline to establish maximal aerobic capacity and evaluate for an adverse response to exercise.

The patient exercise group will be matched with a control group of individuals without known mitochondrial disease who will undergo the same evaluation and training regime (n = 12).

In total, the study will require each participant to attend the research facility for three visits for metabolic examination. The exercise groups will be requested to attend 48 exercise sessions over 16 weeks.

For information about recruitment contact Geoff Bell at [geoff.bell@nuth.nhs.uk](mailto:geoff.bell@nuth.nhs.uk).

## **PHYSICAL ACTIVITY AND INCLUSION BODY MYOSITIS**

**Status: Recruiting**

**Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust**

**Funder: MRC**

**PI: Dr M Trenell**

**Recruitment: 500 recruits expected, across 5 disease sites (all not open yet), stroke arm has 36 recruited, 100 expected**

The aim of this study is to collect data on day to day physical activity levels and metabolic control in individuals with chronic disease.

## DESIGN:

Participants will be identified from chronic disease clinics by the following lead clinicians: Stroke-Prof Gary Ford, Neuromuscular disorders-Prof Kate Bushby, Metabolic disorders-Prof Roy Taylor, fatigue-Prof Julia Newton and Ageing-Prof Julia Newton. An equal sample of male and female participants will be used in the study which will be up to 100 patients in each disease group.

## METHODOLOGY:

Step 1: Relevant practitioners will highlight possible candidates for the study.

Step 2: Visit 1: At the start of the study participants will either be asked to attend Newcastle University's Campus for Ageing and Vitality (Newcastle General Hospital), or if they are an inpatient will be visited on the ward. Participants will be provided with an information sheet about the study. They will be given the opportunity to talk with the team and ask questions. Once fully informed, participants will provide signed informed consent.

Participants will be asked to fill in a disease screening questionnaire at the start of the process. The height and weight of the participants will be recorded and this information will be entered into the physical activity monitors. Instructions will be provided as to how to use the monitors. A resting blood sample may also be taken at this point. This will be analysed for glucose, insulin, lipid profile and liver function.

Step 3: Participants will wear the arm monitors for five days including one weekend day.

Step 4: Visit 2: At the end of the five day period participants will attend the research centre again or attend a pre-arranged session either at their home work place or on the ward to return the activity monitor. Here they will complete a brief physical activity questionnaire and two brief fatigue questionnaires. Data from the physical activity monitor will be fed into a computer. Each participant will be provided with a printout of their weekly activity levels and given the opportunity to discuss their results.

For information about recruitment contact Geoff Bell at [geoff.bell@nuth.nhs.uk](mailto:geoff.bell@nuth.nhs.uk).

## EXERCISE AND SARCOPENIA

**Status: Recruiting**

**Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust Funder: MRC**

**PI: Prof DM Turnbull**

**Patients recruited: 0; target: 36**

**Collaborator Site UCL MRC Centre**

Sarcopenia, which is a complex multifactor process, has significant implications on quality of life, performance of daily activities, maintenance of independence and on projected healthcare costs.

Studies show that low physical activity correlates with poor mitochondrial function. Conversely, exercise correlates with better mitochondrial function, clinical improvement and improved perceived quality of life. Endurance training has been proven to be safe and efficacious in mitochondrial disease which may provide a model for the aging process albeit in an accelerated form with biochemical, histological and genetic changes seen in aged muscle also found in various mitochondrial conditions.

Aims:

- 1.To assess the rate and extent of motor unit loss in the eighth decade of life- cross-sectional (time 0) and longitudinal analysis (end of study)
- 2.To correlate the extent of motor unit loss with histological correlates and the development of sarcopenia
- 3 To assess the impact of exercise on the rate and extent of motor neuron loss

4.To observe whether endurance training initiated in late middle age prevents loss of muscle strength and mass in senescence

5.To assess the impact of neuronal loss on the inability to retain gains made in muscle strength following training after the 7th decade of life

6.To characterise effects of exercise upon neural activity, muscle oxidative capacity and mitochondrial and satellite cell plasticity with age.

Method:

Thirty six (36) female participants, matched for body mass index who do not take regular exercise will be invited to participate: 40- 45 years (12), 60-65 years (12) and 80- 85 years (12). Inclusion criteria will be capacity to undertake cycling exercise and ability to give informed consent. Exclusion criteria will be co-existing active coronary artery disease or steroid therapy.

These patients will be recruited via the media and social support groups. All expenses (travel, accommodation and meals) will be paid for from the research grant.

The study will take place over 24 weeks. Participants will attend the study centre for 7 visits in total. The study will include 2 main visits at the beginning and end of the study. Each main visit will last 3 days. There will also be 5 one day visits.

For information about recruitment contact Geoff Bell at [geoff.bell@nuth.nhs.uk](mailto:geoff.bell@nuth.nhs.uk).

### **Closed Trials**

#### **STRENGTHENING HIP MUSCLES TO IMPROVE WALKING DISTANCE IN PEOPLE WITH CHARCOT- MARIE-TOOTH DISEASE**

**Status: Completed**

**Sponsor: University College London Hospitals**

**Funder: Muscular Dystrophy Campaign (MDC)**

**PI: Dr. Reilly**

**Patients recruited: 32**

Charcot-Marie-Tooth (CMT) disease is a form of hereditary peripheral neuropathy.

People with CMT present with weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles.

The aim of this study will be to investigate the efficacy of a 16 week home based programme of training to increase hip flexor muscle strength and walking endurance. Additional measures of gait speed, exertion, fatigue, disability and general activity will also be recorded. Baseline impairment measures will be obtained to ascertain predictors of strength gains.

This study will use a single blinded, randomised cross over design to investigate if training the hip flexor muscles will strengthen the hip flexor muscle and improve walking endurance in people with all types of CMT.

The trial will include people aged between 18 and 70 years, who have been diagnosed with CMT on the basis of genetic tests (where possible), family history and neurophysiology testing. Each subject will be involved with the study for a 40 week period.

## **Imaging Studies**

### **Set-Up Phase**

None

### **Open Trials**

#### **MRI in IBM and CMT**

Full Title: A Study of Quantitative Magnetic Resonance Imaging and the Clinical Features of Inclusion Body Myositis and Charcot Marie Tooth Disease

**Status: Open to recruitment**

**Sponsor: University College London Hospitals**

**Funder: MRC**

**PI: Prof T Yousry/Dr J Thornton/Prof Reilly/Prof Hanna**

**Patients recruited: 52; target 80**

Magnetic resonance imaging (MRI) is a key tool in the diagnosis and management of a number of diseases. Despite the wide use of MRI in several clinical settings, so far its role in neuromuscular disease has not been well established. The current standard for the diagnosis of neuromuscular disorders includes clinical examination, electrophysiological investigations, biopsy and genetic testing. Due to the nature of the involvement of prominent muscles and peripheral nerves in these disorders it is proposed that MRI could play a prominent role in understanding of neuromuscular disease.

This study aims to investigate the use of MRI as a tool in the study of nerve and muscle diseases by focusing on two particular neuromuscular diseases, one primarily neuropathic and one principally myopathic. Two separate patient cohorts with neuromuscular disease will be recruited. Forty patients with Sporadic Inclusion Body Myositis (IBM) and 40 patients with genetically confirmed Charcot Marie Tooth Disease (CMT). In addition to the two patient cohorts, two groups of healthy volunteers each of size 40 will act as comparators for the disease groups. Each of the patients enrolled in the study will undergo an MRI scanning session in which the quantitative MR techniques developed in Phase 1 with the health volunteers will be applied. In addition to the MRI scanning sessions, each patient will undergo a clinical examination to record the main clinical features of their disease status including an electrophysiological nerve conduction assessment. In the final phase of the study, a sub-group of the patients will then be followed-up at 6 month intervals for 5 years in a longitudinal natural history study of IBM and CMT that focuses on the MR methods and clinical findings that were shown to be most illuminating.

Changes over time in the MRI parameters in the diseased groups and Healthy volunteers will be compared.

Objectives: To detect, using quantitative magnetic resonance imaging (qMRI), the changes in the nerves and muscles of patients with inclusion body myositis or Charcot Marie Tooth disease, and to relate these changes to the measurable clinical and neurophysiological features in these diseases. This will allow the value of various qMRI techniques as markers of disease activity and progression to be tested.

Secondary objectives of the study include: The development of novel quantitative MR techniques for targeted assessment of the human neuromuscular system.

To more fully characterise both the magnetic resonance imaging and clinical features of inclusion body myositis or Charcot Marie Tooth disease as compared with healthy individuals and to study the progression of these characteristics with time over a period of 5 years.

For more information about the study please contact Dr Jasper Morrow at [j.morrow@ion.ucl.ac.uk](mailto:j.morrow@ion.ucl.ac.uk).



## **MRI IN FKRP-RELATED LGMD2I**

Full-Title: A study using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) in Patients with Limb Girdle Muscular Dystrophy 2I; an assessment of muscle damage.

**Status: Open to recruitment**

**Sponsor: Newcastle NHS Trust**

**Funder: MRC**

**PI - Prof V Straub**

**Including cross UK sites-MRC Centre Newcastle and UCL London**

Re-defined in 1995, the LGMDs are face-sparing, proximally predominant, progressive muscular dystrophies with elevated creatine kinase levels and dystrophic features on muscle biopsy. In the current classification system, LGMDs are divided into autosomal dominant (LGMD1) and autosomal recessive (LGMD2) disorders with a superimposed lettering system denoting the chronological order of the chromosomal linkage.

Limb Girdle Muscular Dystrophy 2I (LGMD2I) is caused by a mutation in the fukutin related protein gene (FKRP)<sup>1</sup> and manifests temporal variability. Clinically the age of onset, rate of progression and severity varies greatly between cases and even within the same family. They range from asymptomatic patients with mildly raised creatine kinase levels to those severely affected and non ambulant. The respiratory and cardiac complications, well known to occur in this type of muscular dystrophy, in 30% and 60% of patients respectively, occur independently of the general muscle weakness and also cardiac complications occur independently from respiratory compromise.

Magnetic Resonance imaging (MRI) has been increasingly used in imaging in patients with neuromuscular disorders over the past 5 years.

Studies have shown that whilst there is considerable overlap in muscle involvement there is also striking differences that can be of diagnostic value. In both patients with LGMD2A and LGMD2I there is a prominent pattern of involvement of the posterior thigh muscles, however in LGMD2A there is also selective involvement of the medial gastrocnemius and soleus muscles in the lower leg, which was not seen in LGMD2I. Although it is clearly demonstrated that MRI findings mirror those obtained from clinical examination, it has been reported recently that in fact MRI abnormalities can be detected in patients with neuromuscular disorders when clinical examination of particular muscle groups have been normal. MRI can therefore be useful to show early manifestations of a disease and to monitor the effect of early therapeutic interventions.

Beside MRI another non-invasive technique to consider is phosphorus magnetic resonance spectroscopy (P-MRS). P-MRS studies have demonstrated several metabolic abnormalities in the skeletal muscle of patients with Duchenne Muscular Dystrophy (DMD)/ Becker Muscular Dystrophy (BMD) and in the group of autosomal recessive LGMDs, associated with sarcoglycan deficiency (LGMD2C-F). These changes are thought to be specific for dystrophies secondary to deficits in the dystrophin-glycoprotein complex. In these patients there appears to be an increased cytosolic pH in both groups, however there is also abnormal concentrations of phosphorylated compounds (in particular, decreased phosphocreatine and increased inorganic phosphate concentrations).

The study overall aim is to develop and evaluate non-invasive techniques to quantify muscle pathology and the rate of change over time in LGMD2I, which is potentially a useful tool for monitoring response to treatment and therapies. This shall be achieved by measuring static MRI over a 2 year period and comparing this to age matched adult controls including the quantitative 3-point Dixon technique for measuring fat. At the same time we will also be measuring the Pi and cytosolic pH, ATP and ADP via MRS to see whether a specific pattern of metabolic abnormality is detected in these patients.

For further information about the study please contact Dr. Jasper Morrow at [j.morrow@ion.ucl.ac.uk](mailto:j.morrow@ion.ucl.ac.uk).

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