Fifth UK Neuromuscular Translational Research Conference

22nd – 23rd March 2012

International Centre for Life
Times Square
Newcastle upon Tyne, NE1 4EP

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Registered Charity No. 205395 and
Registered Scottish Charity No. SC039445

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

UK Neuromuscular Translational Research Conference 2012

Dear Colleagues,

We are delighted to welcome you to Newcastle upon Tyne for the fifth 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. Major themes this year include molecular mechanisms in neuromuscular disease, translational pathways, next generation sequencing and stem cells. Dr Robert C. Griggs of the University of Rochester Medical School will deliver the second John Walton lecture.

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.

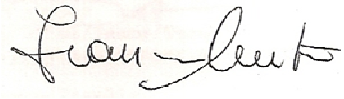
Over the next two days this conference aims to showcase a wide range of high quality scientific neuromuscular research from many UK groups, international colleagues and industry partners.

We have received over 100 high quality abstracts, and there will be dedicated poster sessions each day as well as guided poster discussions. There will be poster prizes for young investigators. All accepted abstracts are published in the journal *Neuromuscular Disorders.*

As the Director, I would very much like to thank the joint MRC-MDC meeting scientific planning team: Professors Kate Bushby, Doug Turnbull, Mary Reilly, Francesco Muntoni, Dame Kay E Davies, 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 Newcastle.

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 Mary Reilly
MRC Centre for Neuromuscular Diseases,
UCL Institute of Neurology

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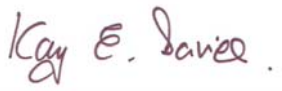
Professor Doug Turnbull
MRC Centre for Neuromuscular Diseases &
Director, Newcastle University Centre for Brain Ageing and Vitality, University of Newcastle upon Tyne



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

Dr Marita Pohlschmidt
Director of Research,
Muscular Dystrophy Campaign

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

Welcome to the 2012 UK Neuromuscular Translational Research Conference organised in partnership between the MRC Centre for Neuromuscular Diseases and the Muscular Dystrophy Campaign.

This is the fifth 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. There is a particular spotlight on how these advances will translate into patient benefit which is what we are all striving so hard to achieve.

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 we continue to lead the fight against all forms of muscular dystrophy and related conditions not only in research but through campaigns, advocacy support, information and advice.

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 secured NHS funding for more than 30 Care Advisor posts across the UK – positions, that over the past 20 years, had 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 in the past 12 months more than 1,000 people living with muscle disease have joined forces with the Muscular Dystrophy Campaign in 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 more than 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 and very unwelcome fact that time is a luxury these patients and families do not have.

I want to 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 here in Newcastle.

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.

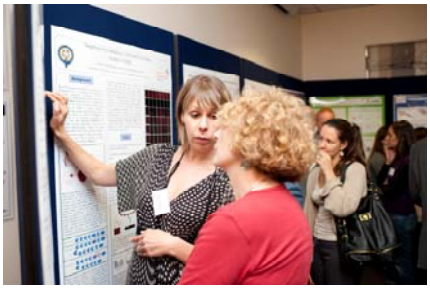
- We recognise the critical importance of training the basic and clinical neuromuscular scientists of the future. The Centre has developed a brand new four-year neuromuscular disease PhD programme, and twelve science PhD students have now been appointed to this programme. We are ensuring that exciting 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 muscular dystrophy and related neuromuscular conditions. 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 these conditions.

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 2012
Scotswood Suite, International Centre for Life, Times Square, Newcastle upon Tyne, NE1 4EP

Day 1 – Thursday 22nd March

09:00 – 10.15	Registration
10:15 – 10:30	Introduction Professor Mike Hanna, UCL Institute of Neurology
10:30 – 12:30	**Molecular Mechanisms in Neuromuscular Disease** **Chair: Professor Doug Turnbull**
10:30 – 11:00	Molecular mechanisms and molecular pathophysiology in Duchenne muscular dystrophy Professor Dominic Wells Professor in Translational Medicine, Royal Veterinary College
11:00 – 11:30	Molecular mechanisms of myofibrillar myopathies Professor Dr Dieter Fürst Institute for Cell Biology, University of Bonn
11:30 – 12:00	Coffee break
12:00 – 12:30	Molecular mechanisms of mitochondrial encephalomyopathies Dr Massimo Zeviani Director, Unit of Molecular Neurogenetics, Fondazione Istituto Neurologico Carlo Besta, Milan
12:30 – 12:45	Platform presentation Dysregulated mitophagy and mitochondrial transport in severe dominant optic atrophy due to OPA1 mutations Professor Joanna Poulton John Radcliffe Hospital, Oxford
12:45 – 13:00	Platform presentation Identification of new chemical compounds which upregulate utrophin for the therapy of Duchenne muscular dystrophy Dr Rebecca Fairclough University of Oxford
13:00 – 14:30	Posters and Lunch, mezzanine
14:30 – 16:30	**Translational Pathways** **Chair: Professor Mary Reilly**
14:30 – 15:00	Navigating the Roadmap – Lessons Learned from Pompe Disease Katherine Klinger Genzyme
15:00 – 15:30	Translating discovery into experimental medicine and treatment - the amyloid experience

Professor Philip Hawkins
Clinical Director, National Amyloidosis Centre,
UCL/Royal Free Hospital NHS Foundation Trust

15:30 – 15:45 Platform presentation
High-dose riboflavin therapy in Brown-Vialetto-Van Laere syndrome: clinical and biochemical improvement
Dr Reghan Foley
UCL Institute of Child Health

15:45 – 16:00 Platform presentation
Efficacy of mexiletine in non-dystrophic myotonia:
results of an international multi-centred randomised controlled trial
Dr Dipa Raja Rayan
UCL Institute of Neurology

16:00 – 16:30 The MRC's translational research strategy
Declan Mulkeen
Director, Research Programmes, Medical Research Council

16:30 – 17:30 Posters & tea, mezzanine

17:30 – 18:30 **The Second John Walton Lecture**
Introduced by Professors Kate Bushby & Doug Turnbull
'Overcoming weakness of the flesh'
Dr Robert C. Griggs, M.D.
Professor of Neurology, Medicine, Pathology, Pediatrics Center for Human Experimental Therapeutics at the University of Rochester School of Medicine and Dentistry, USA

18:30 – 19:30 Drinks reception & posters introduced by Robert Meadowcroft, MDC CEO

20:00 - 22:45 Gala dinner, Scotswood Suite

Day 2 – Friday 23rd March

09:00 – 10:30 Next generation sequencing
Chair: Professor Francesco Muntoni

09:00 – 09:30 Advancing from targeted resequencing to whole exome sequencing:
a perspective on neuromuscular disorders
Professor Madhuri Hegde
Associate Professor/Emory Genetics Lab Scientific Director,
Emory University School of Medicine, USA

09:30 – 10:00 Next generation sequencing in neuromuscular disorders
Professor Henry Houlden
Professor of Neurology, Department of Molecular Neuroscience,
UCL Institute of Neurology

10:00 – 10:15 Platform presentation
Exome sequencing in three families with cytoplasmic body myopathy with early respiratory failure

	Dr Gerald Pfeffer Newcastle University
10:15 – 10:30	Platform presentation tbc
10:30 - 12:45	**Poster guided tours** Mitochondrial disease: Rob Taylor & Mike Hanna Peripheral neuropathies: Rita Horvath & Mary Reilly Neuromuscular MRI: Tarek Yousry Animal models of neuromuscular disease: Volker Straub & Sue Brown Molecular therapies for DMD: Kate Bushby & Nic Wells Congenital myopathies & IBM: David Hilton-Jones & Chris Turner Channelopathies & myasthenia gravis: David Beeson & Hanns Lochmüller Muscle satellite cells: Kay Davies & Jenny Morgan Neuromuscular databases: Adnan Manzur & Matt Parton
12:45 – 13:45	Lunch, mezzanine
13:45 – 15:45	**Stem cells** **Chair: Professor Dame Kay E Davies**
13:45 – 14:15	Epicardium-derived cardiac repair Professor Paul Riley The Oxford Stem Cell Institute, DPAG, University of Oxford
14:15 – 14:45	Stem cells in multiple sclerosis and motor neuron disease Dr Siddharthan Chandran Director of The Euan MacDonald Centre for MND Research & Professor of Neurology, University of Edinburgh
14:45 – 15:15	A phase I/II cell therapy trial for Duchenne Muscular Dystrophy Professor Giulio Cossu UCL Institute of Child Health
15:15 – 15:30	Platform presentation The satellite cell in male and female, developing and adult mice: evidence for functionally distinct stem cell populations Alice Neal UCL Institute of Neurology
15:30 – 15:45	Platform presentation Bmi1 controls satellite cells proliferation and maintenance and plays an important role in muscle regeneration Valentina Di Foggia QMUL, Barts and the London School of Medicine and Dentistry
15:45 – 16:00	Poster prizes and close

Speaker Abstracts

Thursday 22nd March 2012

Molecular mechanisms and molecular pathophysiology in Duchenne muscular dystrophy
Professor Dominic Wells, Royal Veterinary College, London, UK

Molecular mechanisms of filaminopathy, a subtype of myofibrillar myopathy
Professor Dieter O. Fürst, Institute for Cell Biology, University of Bonn, Germany

Myofibrillar myopathy (MFM) is a genetically diverse group of devastating hereditary muscular diseases, manifesting as progressive protein accumulation and myofibril destruction, resulting in premature death. So far MFM has been associated with mutations in seven genes: *BAG3*, *CRYAB*, *DES*, *FLNC*, *FHL1*, *MYOT* and *LDB3*. The conspicuous protein aggregates contain desmin, Z-disc-associated and ectopic proteins.
Until now five mutations in the gene *FLNC*, encoding the large actin-binding protein filamin C, were shown to cause MFM. We have extensively analysed a truncation mutation (p.W2710X) and a 4 amino acid deletion mutation (p.V930_T933del). Expression analyses revealed extensive similarities between muscle biopsies from patients carrying one of these mutations or MFM-causing mutations in other genes. Biochemical and biophysical studies indicated that these mutations directly cause protein folding problems, resulting in the formation of aggregates containing mutant filamin C protein and eventually several other proteins. Transient transfections of mutant filamin C-encoding cDNAs recapitulate the situation observed in patient muscle and have the potential to aid as helpful experimental models for testing future therapeutic strategies.

Molecular mechanisms of mitochondrial encephalomyopathies
Dr Massimo Zeviani MD, PhD, Unit of Molecular Neurogenetics - Neurological Institute C. Besta - via Temolo 4 Milano 20126, Italy

Faulty OXPHOS is the biochemical signature of mitochondrial disorders, a group of highly invalidating human conditions. The genetic and biochemical intricacy of mitochondrial bioenergetics explains their extreme heterogeneity, a formidable challenge for both diagnostic workup and treatment. As a result, only 40% of adult-onset disorders are currently diagnosed at the molecular level, and much lesser so in infantile syndromes. In addition, although energetic failure associated with reduced ATP biosynthesis appears to be critical in individual or combined MRC-complex defects, other mechanisms are also likely to be involved, and are probably predominant in the pathogenesis of specific syndromes, such as alterations of cellular redox status, the production of reactive oxygen species, compromised Ca2+ homeostasis, dysregulation of the proton-motive force and its dissipation, and mitochondrial pathways of apoptosis. By exploiting new technological and biocomputational tools, dedicated databases, and ex vivo experiments, we have identified several new disease genes, each responsible of distinct defects in the assembly and stability of the respiratory chain, in mtDNA maintenance and expression, or in the pathways linking the shape and dynamics of mitochondria with signaling and execution pathways. Structural analysis has allowed us to dissect out the molecular consequences of the ablation or defects of some of the corresponding proteins, and their physical status in normal and disease conditions. To gain further insight on function and mechanism of disease, we have then created specific recombinant lines in yeast, flies, and mice. These models have also been exploited to implement experimental therapeutic strategies, based on gene and cell replacement, or pharmacological control of mitochondrial biogenesis.

Dysregulated mitophagy and mitochondrial transport in severe dominant optic atrophy due to OPA1 mutations
Professor Joanna Poulton, John Radcliffe Hospital, Oxford, UK

Chunyan Liao+, Neil Ashley+, Karl Morten, K Phadwal[1], Andrew Williams[2], Ian Fearnley[2], Lyndon Rosser[3], Jo Lowndes[4], Carl Fratter[5], David Ferguson[6], Laura Vay, Gerardine Quaghebeur[8], Lorna Macleod, Alem Gabriel, Susan Downes, Katja Simon[1] Marcela Votruba[7,8] Joanna Poulton*.
*Nuffield Dept Obstetrics and Gynaecology, The Women's Centre, Oxford OX3 9DU

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+ Equal first authors

OPA1 mutations are the commonest cause of dominantly inherited optic atrophy (DOA), the Opa1 protein being essential for normal mitochondrial fusion. In mouse DOA, autophagy (recycling of spent cellular components) is dysregulated in retinal ganglion cells (RGCs), but mitophagy (mitochondrial recycling) has not been directly implicated.

Profound down-regulation of OPA1 using RNAi caused mitochondrial fragmentation, loss of mitochondrial DNA, impaired mitochondrial function and perinuclear clustering (co-localising with Golgi and microtubule organising centre) in control fibroblasts. We identified a boy with a very severe DOA phenotype, presenting aged 2, developing an axonal sensory ataxic neuropathy and cognitive regression aged 10. Cranial MRI showed very small optic nerves. We identified the well established c.2708_2711delTTAG mutation in the OPA1 gene. He also had a c.661G>A OPA1 variant *in trans*, resulting in a Glu221Lys substitution. Abnormal fibroblast mitochondrial morphology was associated with the c.2708_2711delTTAG mutation.

We validated and used novel ImageStream technology to quantitate mitophagy in primary cells, showing increased co-localisation of mitochondria and autophagomes, consistent with increased mitophagy in the patient's fibroblasts. This was confirmed by western blotting. Ours is the first example of abnormal mitophagy in a human genetic disease. Furthermore, genetic background appears to influence penetrance.

We suggest that OPA1 mutations cause constitutive mitochondrial fragmentation and dysregulated mitophagy. When OPA1 is profoundly reduced, increased mitophagy causes mitochondrial DNA depletion. RGCs may be particularly susceptible to mitochondrial DNA depletion and to impaired plus ended microtubule mediated transport, both of which may impair the supply of energy to sites of high energy usage.

Identification of new chemical compounds which upregulate utrophin for the therapy of Duchenne muscular dystrophy
Dr Rebecca Fairclough, University of Oxford, UK

Rebecca. J. Fairclough†, Sarah E. Squire†, Allyson C. Potter†, Dave S. Powell†, Stephen G. Davies*****, Carole J. R. Bataille*, Graham M. Wynne*, Angela J. Russell*****¶ and Kay E. Davies†
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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. In partnership with Summit plc, our previous drug screen lead to the development of SMT C1100 – the first drug to enter clinical trials based on utrophin upregulation for the therapy of DMD. This provided crucial proof-of-principle for the strategy we have developed. We now aim to build on our previous results and perform a new drug screen based on improved *in vitro* and *in vivo* screening tools. A new screening assay based on immortalised myoblasts from the utrophin luciferase (*LUmdx*) knock-in mouse model enables us to screen the utrophin promoter in its genomic context. This should better mimic the *in vivo* situation and also enable identification of compounds which upregulate utrophin

through regulatory pathways outside of the 8.4 kb promoter A fragment that formed the basis of our previous screen. Compounds (7000) have been specifically selected from a 25000 member library to enhance the hit rate whilst screening substantially fewer compounds. Selection criteria included similarity, yet structural distinction, to known utrophin upregulators, along with chemical scaffolds containing motifs of known transcriptional activators. Screening has identified new structural classes with utrophin upregulating capabilities together with compounds demonstrating enhanced activity compared to our current positive controls.

Navigating the Roadmap – Lessons Learned from Pompe Disease
Katherine Klinger, Genzyme, Ma, USA

Translational medicine is often described as 'the path from bench to bedside" with the goal of decreasing the time required to move new discoveries into the clinic, and increasing the likelihood of therapeutic success. Simple in concept, the process can be extremely challenging in practice, as illustrated by our experience in developing therapy for Pompe disease.
Pompe disease is a genetic disease resulting from a deficiency of lysosomal acid alpha-glucosidase (GAA). Inherited in an autosomal recessive manner, the disease can manifest as a clinical spectrum with regard to disease severity and progression based on the amount of residual GAA activity. In terms of translational medicine, Pompe disease would seem to be an ideal candidate. The underlying molecular lesion was known (lack of GAA) and the therapeutic entity was clear (enzyme replacement). In fact, answering key translational questions such as "how well do we understand the disease", and "how well do we understand the mechanism of action of the drug" revealed a number of hurdles that had to be overcome along the way.
Enzyme replacement therapy (ERT) for Pompe disease was approved by the FDA in 2006, and it has been more than ten years since the first patients were treated in clinical trials. ERT has had a dramatic impact on patient outcome. However, new discoveries continue, as the phenotype of treated patients emerges. Thus the path is 'back from the bedside to the bench', in the search for optimal patient care.

Translating discovery into experimental medicine and treatment – the amyloid experience
Professor Philip Hawkins, UCL/Royal Free Hospital NHS Foundation Trust, London, UK

Accumulation of amyloid fibrils in the viscera and connective tissues causes systemic amyloidosis, which is responsible for about one in a thousand deaths in developed countries. Localized amyloid can also have serious consequences; for example, cerebral amyloid angiopathy is an important cause of haemorrhagic stroke. The clinical presentations of amyloidosis are extremely diverse and the diagnosis is rarely made before significant organ damage has occurred. There is therefore a major unmet need for therapy that safely promotes the clearance of established amyloid deposits. Over 20 different amyloid fibril proteins are responsible for different forms of clinically significant amyloidosis, and treatments that substantially reduce the abundance of the respective amyloid fibril precursor proteins can arrest amyloid accumulation. This approach has included liver transplantation in familial amyloidotic polyneuropathy to remove the hepatic source of genetically variant, amyloidogenic forms of transthyretin. Unfortunately, control of fibril-protein production is not possible in some forms of amyloidosis, and in others it is often slow and hazardous.
There is no therapy that directly targets amyloid deposits to enhance their clearance. However, all amyloid deposits contain the normal, non-fibrillar plasma glycoprotein, serum amyloid P component (SAP), which we have exploited at the National Amyloidosis Centre for 20 years as the basis for radiolabelled SAP scintigraphy, a quantitative imaging method for diagnosis and monitoring of systemic amyloid deposits. We have more recently investigated the potential of SAP as a therapeutic target. SAP is universal in all amyloid deposits, in which location it is not degraded. The binding of SAP promotes fibrillogenesis and stabilises amyloid fibrils in-vitro. We demonstrated that SAP knockout mice show retarded, reduced amyloid deposition, and developed a drug, CPHPC, that is a competitive inhibitor of SAP binding to amyloid fibrils. This palindromic compound crosslinks and dimerizes SAP molecules, leading to their very rapid clearance by the liver, and thus produces marked depletion of circulating human SAP.
This unanticipated new pharmacologic phenomenon of specific plasma protein depletion opened up the possibility of an immunotherapeutic approach using anti-SAP antibodies that can then reach

residual SAP in the amyloid deposits. In an experimental systemic AA amyloidosis model, the combination of CPHPC and anti-SAP therapy triggers a potent, complement-dependent, macrophage-derived giant cell reaction that swiftly removes massive visceral amyloid deposits without adverse effects. The unprecedented capacity of this novel combined therapy to eliminate amyloid deposits should be applicable to all forms of systemic and local amyloidosis, and is currently the subject of a fully collaborative clinical development programme with GlaxoSmithKline.
Other therapies for amyloidosis that have already entered clinical testing include treatments developed to stabilize and maintain circulating amyloid precursor proteins in their normal confirmation, agents that inhibit the interaction between amyloid fibrils and glycosaminglycans, other immunotherapy approaches, and small interfering RNA and antisense oligonucleotide technologies. These various new therapies offer real promise that effective anti-amyloid treatment will finally become available within the next few years.

High-dose riboflavin therapy in Brown-Vialetto-Van Laere syndrome: clinical and biochemical improvement
Dr Reghan Foley, UCL Institute of Child Health, London, UK

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Background: Brown-Vialetto-Van Laere syndrome (BVVL) was first described in 1894; however, the full range of clinical phenotypes and the pathophysiology of this complex neurodegenerative disorder have not been well-delineated. A biochemical profile similar to that of multiple acyl-CoA dehydrogenase deficiency (MADD), due to dysfunction of the riboflavin-dependent enzymes in the mitochondrial electron transfer flavoprotein chain, has been reported in patients with BVVL. Recently, the identification of a gene coding for a putative riboflavin transporter has been identified in patients with BVVL syndrome. We report the case of a patient with a clinical phenotype suggestive of BVVL syndrome and a biochemical profile mimicking MADD who has mutations in a newly identified gene whose function is postulated to be that of riboflavin transport.
Results: After three months of riboflavin supplementation with daily doses up to 150 mg, our 10 year-old patient with sensory motor neuropathy, sensorineural hearing loss, optic neuropathy and sleep hypoventilation has demonstrated clear improvements on audiometry and pulmonary function testing and a normalisation of her acylcarnitine and urine organic acid profiles.
Conclusions: BVVL syndrome, hitherto without a viable treatment option, can demonstrate clinical and biochemical improvement on a regimen of high-dose riboflavin therapy, in particular when mutations are present in putative riboflavin transporter genes. In neurodegenerative conditions the translation of our understanding of disease mechanisms into treatments which can affect objective clinical improvement is exceedingly rare. It is, therefore, essential that patients presenting with BVVL syndrome be tested for mutations in riboflavin transporter genes and offered riboflavin therapy.

Efficacy of mexiletine in non-dystrophic myotonia: results of an international multi-centred randomised controlled trial
Dr Dipa Raja Rayan, UCL Institute of Neurology, London, UK

D.L. Raja Rayan[1], E. Matthews[1], G. Barreto[1], S.V. Tan[1], L. Dewar[1], J. Burge[1], Y. Wang[2], J.Trivedi[3], E.Ciafaloni[4], M. Salajegheh[5], S.Venance[6], G.Meola[7], B.Bundy[8], L. Herbelin[2], J. Statland[4], R. Griggs[4], R. Barohn[2], M.G. Hanna[1] and the CINCH group
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The Non-Dystrophic Myotonias (NDM) are a group of hereditary skeletal muscle channelopathies caused by mutations in either the chloride channel, *CLCN1* or sodium channel, *SCN4A* gene. They can cause disabling stiffness, fatigue and weakness and therefore often need treatment. There is anecdotal evidence that mexiletine may reduce stiffness in these patients but no randomised, double-blind, placebo-controlled trials have been done to date.

We conducted an international, multi-centred, randomised, double-blind placebo-controlled, crossover trial of 59 subjects with NDM. Participants were randomised to mexiletine 200 mg tds or placebo for 4 weeks, followed by a 1 week wash out, and then crossover to the alternative treatment for 4 weeks. Efficacy of the drug was monitored by daily patient reported symptom severity (stiffness, pain, weakness, tiredness) on a scale of 1-9. Improvement in stiffness was the primary outcome measure. Secondary outcome measures included clinical assessment, quality of life, quantitative grip myotonia assessment and neurophysiological assessment.

Mexiletine was found to significantly improve stiffness compared with placebo with a mean difference of 2.73 ($p<0.0001$). It also significantly improved pain, weakness and fatigue. Quality of life measures showed a significant improvement in almost all areas, especially bodily pain (SF-36) and muscle locking (INQoL) and both clinical and quantitative handgrip assessment showed a significant difference. Mexiletine was well tolerated with no serious cardiac events. The main side effects were gastrointestinal, reported in 15%.

Mexiletine is therefore a safe and effective drug in the treatment of stiffness in NDM and should be considered as a first line treatment.

The MRC's translational research strategy
Mr Declan Mulkeen, Medical Research Council, London, UK

The Second John Walton Lecture: Overcoming weakness of the flesh
Dr Robert C. Griggs MD, University of Rochester School of Medicine, USA

Friday 23rd March 2012

Advancing from targeted resequencing to whole exome sequencing: a perspective on Neuromuscular disorders
Professor Madhuri Hegde, Department of Human Genetics, Emory University School of Medicine, USA

A comprehensive approach to identifying the causative gene and understanding the underlying mechanism associated with each disease genotype is inevitable to diagnose disease and eventual selection of effective therapeutic strategy. Recent advances in genomic technologies have enabled rapid identification of new genes and developing novel diagnostic strategies for known and unknown forms of muscular dystrophies by whole exome and genome sequencing and targeted gene panels. Mutations in a single gene and altered levels of the corresponding protein may further alter the expression pattern of closely related proteins, especially in the case of muscle proteome where several proteins form structural complexes, thereby modifying of the severity the disease phenotype and presenting overlapping features. Therefore, a comprehensive approach and confirmatory studies will help delineate the disease subtypes. This presentation will highlight the power of combining WES with functional assays to identify the new genes causative of muscular dystrophy.

Next Generation Sequencing in Neuromuscular Disorders
Professor Henry Houlden, UCL Institute of Neurology, London, UK

Neuromuscular disorders are genetically extremely heterogeneous and there are often few diagnostic clues to indicate the defective gene. Next generation sequencing (NGS) offers a comprehensive route to identify these genes. There are currently two NGS methods that we are employing in neuromuscular and other neurogenetic disorders:

1. Gene panels. Here we have developed panels of disease genes that are designed based on specific amplicons for gene exons, enriched and sequenced on the Illumina MiSeq. This allows us to sequence the genes we want at a high coverage level of 100-200 fold. We are currently testing panels for recessive and dominant neuropathy genes, episodic neurological disorders such as EA1 and 2 and the muscle channel genes such as CLCN1.
2. Exome sequencing. Where families or specific patients are negative for the known neuromuscular genes we are sequencing one or two probands to identify new disease genes. This is difficult as a tremendous amount of data is produced from each exome run and there are often many potential disease variants. There are a number of ways to filter data, such as by expression and putative gene function but one of the best ways is to compare variants from other affected cases with the same phenotype.

In this presentation I will go over this technology, uses and limitations using examples of the data we have produced as well as discuss the steps that we wish to take to make data accessible to over groups and combine exome datasets.

Exome sequencing in three families with cytoplasmic body myopathy with early respiratory failure
Dr Gerald Pfeffer, Newcastle University, UK

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Introduction: Cytoplasmic body myopathy with early respiratory failure (CBM) is a rare muscular dystrophy characterised by the presence of nonspecific muscle pathology, cytoplasmic bodies on electron microscopy, eosinophilic inclusions on muscle histopathology, and the clinical presentation of early respiratory failure (while still ambulant) in patients with distal and/or proximal muscle weakness. In the world literature the genetic defect for this condition has been identified in two families, having the R279W mutation in the sarcomeric protein *TTN*, although cases without this mutation have already been reported.
Methods: Two large pedigrees and an apparently sporadic case with CBM were included in this study. Clinical characteristics and diagnostic investigations of all affected family members was collected from charts and summarised. Western blot of C-terminal *TTN* protein was performed. Genome-wide 1000 microsatellite analysis was performed in one of the families to identify a linkage region and shared haplotype. Exome sequencing was performed on 4 patients.

Results: Onset of disease was usually in middle-age, with tibialis anterior being the earliest and most severely affected muscle. Pulmonary function tests indicated low FEV1 and FVC, with worsening when supine. Cardiac involvement was not present. Muscle pathology was nonspecific in some affected patients. Electromyography demonstrated necrotising myopathic process in the majority of patients. MRI invariably revealed signal abnormalities of semitendinosus muscle. Blot of C-terminal *TTN* protein was normal. A single 20 cM linkage region on chromosome 2 was identified. Exome sequencing detected the disease mutation and identified other candidates for exclusion with segregation analysis.
Conclusions: We report the disease gene for these patients with CBM, and describe in detail the phenotype and diagnostic investigations from these three pedigrees. Genetic testing for these CBM

genes should be performed in patients with myopathy and early respiratory failure if muscle pathology reveals cytoplasmic bodies or is nonspecific.

Acknowledgements: GP is the recipient of funding from the Clinician Investigator Program from the University of British Columbia in Vancouver, Canada, and a Bisby fellowship from the Canadian Institutes of Health Research. The authors report no competing interests.

Epicardium-derived cardiac repair
Professor Paul Riley, University of Oxford, UK

We adopt the paradigm of understanding cardiovascular development as a first principal to inform on adult heart repair and regeneration. Thymosin β4 (Tβ4) is a small actin monomer binding protein which we previously demonstrated is both necessary for the development of epicardial-derived coronary vasculature and sufficient to activate quiescent adult epicardial cells (EPDCs). Tβ4 treated EPDCs adopt an embryonic fate and contribute vascular smooth muscle cells to neovascularisation of both the intact and injured adult heart. During development EPDCs also contribute cardiomyocytes to the forming heart muscle. Recently we sought to extrapolate this myocardial potential to the adult heart. In the absence of markers specific for the adult epicardium, we primed $Wt1^{CreGFP/+}$ and $Wt1^{CreERT2/+};R26R^{EYFP/+}$ mice with Tβ4, followed by myocardial infarction, to reactivate Wt1 in the adult lineage and enable GFP or YFP (+tam) -labeling of EPDCs. Priming initiated an embryonic gene programme in the adult progenitors, and subsequent tracking of their fate, in response to injury, revealed a small subpopulation which migrated inward and contributed *de novo* cardiomyocytes at the border zone of the infarct. Transplantation of labeled donor cells into unlabelled hosts confirmed a progenitor origin for the cardiomyocytes and FISH revealed they formed in the absence of fusion with host myocytes. EPDC-derived cardiomyocytes structurally and functionally coupled with surviving resident myocardium and contributed, in part, to improved cardiac function, as a significant step towards myocardial regeneration and heart repair.

This work was generously supported by the British Heart Foundation.

Stem cells in multiple sclerosis and motor neuron disease
Dr Siddharthan Chandran, University of Edinburgh, UK

A phase I/II cell therapy trial for Duchenne muscular dystrophy
Professor Giulio Cossu, UCL Institute of Child Health, UK

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Mesoangioblasts are recently characterized progenitor cells, associated with the vasculature and able to differentiate into different types of solid mesoderm, including skeletal muscle (Minasi et al. Development 129, 2773, 2002). When mesoangioblasts were delivered intra-arterially to muscles of dystrophic mice and dogs they resulted in a significant functional amelioration (Sampaolesi et al. Science 301, 487, 2003; Nature 444, 574, 2006). Human adult mesoangioblasts, isolated and expanded in vitro from muscle biopsies, were shown to correspond to a subset of pericytes (Dellavalle et al. Nature Cell Biol. 9, 255, 2007).

Based on these results, a monocenter, prospective, non-randomised, clinical phase I/II study of cell therapy with HLA-matched donor human mesoangioblasts in DMD patients started in June 2009, with a one year preliminary study (involving 28 DMD patients, aged 5-10), required to validate outcome measures. Starting on March 2011, three out of these patients (with an HLA-identical donor) underwent successive intra-arterial transplantations at escalating doses of cells, under a continuous regime of immune suppression. Safety was the primary objective of the study. A possible

increase in muscle strength as a consequence of mesoangioblast transplantation will also be evaluated.

Limitations of the current strategy and possible solutions to overcome them will also be discussed.

This work is supported by grants from the European Community (Optistem), the European Research Council, Duchenne Parent Project, Telethon, CureDuchenne, AFM and the Italian Ministries of Research and Health.

The satellite cell in male and female, developing and adult mice: evidence for functionally distinct stem cell populations
Ms Alice Neal, UCL Institute of Neurology, UK

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Satellite cells are myogenic cells found between the basal lamina and the sarcolemma of the muscle fibre. Satellite cells are the source of new myofibres; as such, satellite cell transplantation holds promise as a treatment for muscular dystrophies. We have investigated age and sex differences between mouse satellite cells *in vitro* and assessed the importance of these factors as mediators of donor cell engraftment in an *in vivo* model of satellite cell transplantation. We found that satellite cell numbers are increased in growing compared to adult and in male compared to female adult mice. We saw no difference in the expression of the myogenic regulatory factors between male and female mice, but distinct profiles were observed according to developmental stage. We show that, in contrast to adult mice, the majority of satellite cells from two week old mice are proliferating to facilitate myofibre growth; however a small proportion of these cells are quiescent and not contributing to this growth programme. Despite observed changes in satellite cell populations, there is no difference in engraftment efficiency either between satellite cells derived from adult or pre-weaned donor mice, male or female donor cells, or between male and female host muscle environments. We suggest there exist two distinct satellite cell populations: one for muscle growth and maintenance and one for muscle regeneration.

Bmi1 controls satellite cells proliferation and maintenance and plays an important role in muscle regeneration
Ms Valentina Di Foggia, Queen Mary University of London, UK

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The Polycomb group protein Bmi1 plays an important role in the regulation of adult stem cells self-renewal, maintenance and proliferation. Here we show that both human and murine satellite cells express Bmi1. Specifically, Bmi1 is expressed in both the "stem" Pax7+;Myf5- and "progenitor" Pax7+;Myf5+ satellite cells population.

Here we show that even if Bmi1 is dispensable for embryonic myogenic development, its loss (Bmi1-/- mice) leads to a depletion of Pax7+;Myf5- cells with reciprocal increase in committed myogenic progenitors postnatally. Although Bmi1-/- mice have an increased number of smaller fibers, there are no differences in muscle pattern, composition and muscle strength in comparison with control littermates. Conversely, upon freeze injury, the regeneration of the muscle is delayed: Bmi1 -/- mice show more small centrally nucleated fibres and a larger proportion of embryonic MyHC positive fibres 10 days after freeze injury, suggesting a crucial role of Bmi1 in muscle regeneration in a traumatic setting.

Ablation of Bmi1 leads to affected muscle regeneration in a chronic injury environment too. Mdx;Bmi1-/- mice (the *Mdx* is a mouse model for the human Duchenne Muscular Dystrophy) show reduced number of regenerating fibers and increased number of smaller fibers in the diaphragm, which is one of the muscles particularly affected in this disease.

In keeping with these findings, Bmi1-/- satellite cells in culture show reduced proliferation and a failure in re-entering the cell cycle after stimulation with high concentration of serum, suggesting the important role of Bmi1 in the maintenance of the stem cell pool of the postnatal skeletal muscle.

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Poster Abstracts

DMD – Molecular Therapy

Poster 1

Correlation of internally deleted dystrophin and dystrophin-associated protein expression with clinical severity in Becker muscular dystrophy

Karen Anthony[1], Sebahattin Cirak[1], Silvia Torelli[1], Giorgio Tasca[2], Lucy Feng[1], Virginia Arechavala-Gomeza[1], Annarita Armaroli[3], Michela Guglieri[4], Chiara Straathof[5], Jan Verschuuren[5], Annemieke Artsma-Rus[6], Paula Helderman-van den Enden[6], Katherine Bushby[4], Volker Straub[4], Caroline Sewry[1], Alessandra Ferlini[3], Enzo Ricci[7], Jennifer Morgan[1], Francesco Muntoni[1].

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Exon skipping using antisense oligonucleotides represents a potential effective disease modifying treatment in Duchenne muscular dystrophy (DMD). However there is debate regarding the functional properties of the internally deleted dystrophins produced by exon skipping and whether the skipping of some exons is more beneficial than others. In order to better predict the outcome of exon skipping clinical trials we have characterised the clinical phenotype of 17 Becker muscular dystrophy (BMD) patients harbouring in-frame deletions relevant to on-going or planned exon skipping clinical trials for DMD and correlated it to the levels of dystrophin, and dystrophin-associated protein expression. Patients were selected exclusively on the basis of their genotype; all were classified clinically as BMD and were grouped into asymptomatic (4 patients), mild (12 patients) and severe (1 patient) categories. All 17 patients had dystrophin levels of at least 40% of control with the asymptomatic group having significantly higher dystrophin ($p = 0.013$), β-dystroglycan ($p = 0.025$) and neuronal nitric oxide synthase (nNOS, $p = 0.034$) expression compared with symptomatic BMD patients. Furthermore, patients with deletions ending in exon 51 (whose skipping could rescue the largest group of DMD deletions) showed significantly higher dystrophin levels ($p = 0.034$) than those with deletions ending with exon 53. This is the first quantitative study on both dystrophin and dystrophin-associated protein expression in BMD patients with deletions relevant for on-going and future DMD exon skipping clinical trials. Taken together our results indicate that a significant clinical benefit from the production the internally deleted dystrophins assessed in this study is a realistic possibility.

Poster 2

Development of *in vivo* imaging techniques to determine the biodistribution of antisense oligonucleotides in dystrophin deficient muscular dystrophy

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Antisense-oligonucleotide (AON) induced gene skipping is one of the most promising strategies for treating Duchene muscular dystrophy [1], with the first generation of AONs advancing to phase 3 clinical trials [2]. These AONs have been shown to induce specific gene skipping leading to increased dystrophin expression and muscle function *in vivo*. However, the efficacy of these AONs is limited by their poor pharmacokinetic properties, including poor targeting and uptake by key affected tissues such as the heart and diaphragm [3]. Therefore, the next generation of AONs have been conjugated to cell penetrating peptides (CPP), which have shown early promise in pre-clinical studies. Several studies have already demonstrated enhanced pharmacokinetic properties of conjugated AONs together with improved tissue targeting and an overall increase in efficacy [4]. Interestingly the *in vivo* activity of conjugated AONs is sensitive to even small changes in the sequence of CPPs; therefore

several modified sequences have been generated for pre-clinical evaluation[5]. However, the optimisation of these peptides is currently limited by the lack of non-invasive methods for evaluating the changes to biodistribution profiles *in vivo*. One promising method is the addition of a small radioactive ^{18}F ligand to the conjugated AON and using PET imaging to monitor and evaluate the biodistribution of AONs *in vivo*. The aim of this project is to develop a robust PET imaging platform for determining the biodistribution and pharmacokinetics of conjugated AONs in mdx mice. This will provide essential information regarding the possible efficacy and toxicity of the conjugated AONs, which can be extended in to clinical studies.

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Poster 3

What do MHCn and MHCd antibodies recognise?

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Muscular dystrophies and many forms of congenital myopathies have increased numbers of fibres with fetal/neonatal myosin and/or developmental myosin. Immunohistochemical labeling of immature isoforms of myosin heavy chains has been widely used for determining muscle regeneration. However the protein detected by the antibodies often used (fetal/neonatal, MHCn and developmental, MHCd) has not been fully established
The immunohistochemical results of patient muscle biopsies with various neuromuscular conditions were reviewed. The number and pattern of positive fibres recognised by MHCn and MHCd were compared.
Both MHCn and MHCd clearly label basophilic fibres in dystrophic muscle. There are often more MHCn-positive fibres than MHCd-positive fibres, but not all positive fibres are basophilic. Most fibres positive for MHCn and negative for MHCd are of larger diameter with variable intensity of labelling. In addition there are variations between fascicles. Atrophic nuclear clump fibres label with MHCn but rarely with MHCd. Groups of fibres positive with MHCn are also present in cases with denervation (e.g. SMA), and cases with minimal pathology may show positive fibres of normal diameter with MHCn.
Thus MHCn not only recognizes regenerating fibres but also fibres that are abnormal, some which may be denervated.

Poster 4

Advancing the potential of peptide-PMO compounds in exon skipping therapy for DMD

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Duchenne muscular dystrophy (DMD) is an incurable, fatal muscle degenerative disease caused by mutations in the *DMD* gene that result in an absence of dystrophin protein. Modulation of *DMD* pre-mRNA splicing by exon skipping to bypass out-of-frame deletions and premature stop codons, is currently the most promising molecular intervention to restore dystrophin protein expression.

However this approach is complicated by the relatively inefficient skeletal muscle delivery of antisense oligonucleotides as well as the inadequate targeting of the heart. Recent studies have highlighted the potential of peptide conjugated phosphorodiamidate morpholino oligonucleotide (PMO) compounds to improve systemic delivery and thus advance the development of exon skipping therapies for DMD. In order to understand and improve upon the activity of peptide-PMOs, we have investigated dosing regimens, routes of systemic delivery and compound formulation to devise the optimal delivery regimen for these compounds. Here we report the results of a repeat low dose study performed using Pip5e-PMO, B-MSP-PMO, B-PMO and PMO in the *mdx* mouse. We also report our findings following a route optimisation study performed to compare efficacy of Pip6e-PMO, B-MSP-PMO, B-PMO and PMO following either intravenous tail vein or subcutaneous injections. In addition we demonstrate the relative efficacy of Pip6e-PMO formulated in saline, glucose or intralipid following systemic injection. We show that peptide-PMO technology has significant potential for overcoming the major systemic and tissue specific delivery challenges associated with antisense oligonucleotide therapy.

Poster 5

Assessment of potential promoters for lentiviral gene therapy in DMD

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Duchenne Muscular Dystrophy (DMD) is a progressive X-linked genetic disorder characterised by an early onset of muscle degeneration due to the absence of functional dystrophin protein. Damaged muscle fibres can initially be regenerated by a muscle-resident stem cell population called satellite cells (SCs), which underlie the myofibre basal lamina. Following activation, SCs proliferate extensively and fuse with the damaged fibre, however, some SCs return to quiescence to maintain the stem cell pool. However, regeneration eventually fails and the muscle becomes progressively wasted, resulting in premature death of the patient. Long-term correction of dystrophic muscle and SCs requires the insertion of a functional dystrophin gene copy into the mutated genome. Lentiviral vectors (LVs) represent suitable candidates for DMD gene therapy, as they stably integrate their genome into dividing and non-dividing cells, thereby mediating long lasting expression in both proliferating myoblasts and post-mitotic myofibres. Importantly, a careful choice of promoter is necessary to avoid off-target transduction and to circumvent associated safety concerns due to non-physiological expression levels.
This study compares LVs carrying either a strong viral, a muscle-specific or a housekeeping promoter, and assesses variances in expression levels, integrated copy numbers and persistence of transgene expression. Tissue-specificity of promoters will be discussed. Transgene expression mediated by randomly integrated viral copies did not alter the potential of SCs to differentiate and self-renew *in vitro* and *in vivo.* Notably, human myoblasts showed significantly enhanced transduction efficiencies compared to murine myoblasts, thus underpinning the great translational potential of LVs in the area of muscle degenerative diseases.

Poster 6

Audit of unexpected weight loss in patients with Duchenne muscular dystrophy

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Background Whilst a significant proportion of individuals affected by Duchenne muscular dystrophy (DMD) are overweight, a number are at risk of weight loss with disease progression. Poor nutrition can potentially have negative effect on every organ system and can contribute to reduced life expectancy.
Objective: To identify the causes for weight loss in this patient group as a potential marker for deterioration in organ systems that might be amenable to targeted intervention.

Method: Included in this retrospective audit were individuals affected by DMD 10 years or older treated at our centre alone over the last 5 years, who experienced weight loss. Descriptive statistics used to analyse the data in view of small numbers.
Results: We identified 19/77 (24.7%) patients who experienced 30 episodes of weight loss. The average percentage weight loss was 6.29%. Weight loss was attributed to various reasons: respiratory deterioration (10%), cardiovascular deterioration (6.6%), infections (6.6%), combined problems of respiratory, cardiovascular and gastroenterological deterioration (30%), worsening scoliosis (3.3%), post surgery (13.3%) and deliberate weight loss (6.6%). Left ventricular fractional shortening and sitting forced vital capacity (FVC) six months prior and at time of weight loss showed no change in fractional shortening in 90% and 43% had reduction of FVC by an average of 7.7%. The average time to regain weight was 7.6 months. 43% episodes had weight loss of >5% and 70% took longer than 3 months to regain weight.
Conclusion: Unexpected weight loss in this patient group might be more frequent than expected with almost 25% in our group. Our study shows that majority of patients with DMD combined respiratory, cardiovascular and gastroenterological causes were responsible for weight loss and most patients took longer to recover. Unexpected weight loss can be a marker for potential significant respiratory and/ or cardiac deterioration prompting targeted investigations and supportive management.

Poster 7
Meganuclease-Enhanced Genome Correction Therapy for Duchenne Muscular Dystrophy
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Duchenne muscular dystrophy (DMD) is a severe inherited, muscle-wasting disorder caused by mutations in the dystrophin gene. This study is an investigation into gene correction of human deletions in cultured myogenic DMD cells using meganucleases (MN), a synthetic target-specific DNA endonuclease, together with a specific repair matrix. This is the first report of using genome surgery for dystrophin gene correction in human patient cells, and suggests exciting therapeutic potential for the treatment of DMD patients.
Meganucleases were designed to cut within intron 44, upstream of a deletion hot-spot within the dystrophin gene which accounts for 65% of DMD-causing mutations. Integration-competent (ICLV) and integration-deficient (IDLV) lentiviral vectors expressing the MN were generated. Western blotting and deep gene sequencing was used to establish MN expression and activity. A repair matrix carrying exons 45-52 was designed and packaged into IDLV. Co-infection of LV-MN and LV-targeting matrix into immortalised del45-52 patient cells was performed. RNA and genomic DNA were analysed to establish exonic knock-in and gene correction.
Expression of fully corrected dystrophin RNA was observed. Genomic DNA showed that homologous recombination between endogenous genomic DNA and the repair matrix had occurred in response to the cut made by target-specific MN. Our data show that use of a MN in conjunction with a repair matrix results in successful gene correction. Such an approach is expected to result in stable expression of full-length dystrophin protein. This novel approach may lead to the development of a permanent gene correction therapy for DMD.

Poster 8
Restoring the reading frame in large DMD duplication mutations results in dystrophin expression *in vivo*
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Antisense oligonucleotide (AON) therapy has been shown to be an effective treatment for restoring dystrophin expression in mdx mice and Duchenne muscular dystrophy (DMD) patients with out-of-frame mutations. However, to date, limited exon skipping studies have been conducted in DMD patients with large out-of-frame duplication mutations. Whilst exon-skipping one or two exons is a highly efficient process in cell culture, efficiency falls when targeting multiple exons, thus producing little of the desired end product Therefore, we sought to understand the expression and function of large DMD duplication proteins when only two exons are removed in order to restore the reading frame. Here we construct large "in-frame" DMD duplication mutation plasmids and evaluated their expression *in vivo.*
Two out-of-frame DMD duplication mutations (dup ex22-29 and dup ex18-30) were constructed to express in-frame human dystrophin transcripts (del ex21-22 dup ex23-29 and del ex17-18 dup ex19-30). In addition, an original "in-frame" DMD duplication mutation (dup ex3-25) was constructed to determine if over expression of the duplicated transcript would result in protein expression. Plasmids were electroporated into TA muscles of *mdx* mice, and human dystrophin expression was evaluated 7 days later. Human dystrophin expression was detected at the sarcolemma for all the constructs, confirming the duplicated proteins were stable. In addition, all three proteins recruited the nNOs protein.
Thus DMD patients with out-of-frame duplication mutations may be suitable candidates for AON therapy. Further work to determine if the duplicated proteins provide protection against muscle damage during exercise is currently underway.

Poster 9
Novel 5'-utrophin isoforms exhibit species-specific transcriptional profiles and provide additional candidates for therapeutic up-regulation in DMD
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Duchenne muscular dystrophy (DMD) is a fatal X-linked childhood disease caused by absence of dystrophin at the muscle membrane. Dystrophic symptoms are preventable in the *mdx* mouse model by over-expression and sarcolemmal redistribution of the autosomal paralogue utrophin; an attribute that has been the intense focus of up-regulation based therapeutic strategies for DMD. Clues as to the potential existence of additional utrophin-based targets arise from the observation that the severe muscle phenotype characteristic of DMD is comparable only in mice lacking both utrophin and dystrophin, implying the mouse utrophin locus contains uncharacterised transcriptional attributes that allow functional compensation. To investigate this phenotypical disparity, we performed comprehensive analysis of mouse and human utrophin loci and report the identification of multiple novel 5' isoforms, including conserved alternatives to previously identified full-length transcripts (utrn-A and –B), two specific to mouse and one conserved in both species. Each isoform exhibits distinctive transcriptional profiles in tissue, embryogenesis, myogenesis and in mesoangioblast stem cells. Crucially, individual isoforms also illustrate clear speciation differences in the absence of dystrophin, providing mechanistic insight into how utrophin may contribute to the more effective *mdx* response in comparison to DMD. This study thus highlights a previously unexplored approach of "mimicking" aspects of the utrophin-related response to dystrophin deficiency in mice for therapeutic benefit in humans.

Poster 10
The next DMD exon skipping trial: selection of AO target
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Duchenne muscular dystrophy (DMD) is caused by the lack of dystrophin protein, most commonly as a result of frame-shifting mutations, both deletions and duplications, in the dystrophin gene. Selective removal of exons flanking an out-of-frame DMD mutation can result in an in-frame mRNA transcript that may be translated into an internally-deleted, Becker muscular dystrophy (BMD)-like but functionally active dystrophin protein with therapeutic activity. Antisense oligonucleotides (Aos) have been designed to bind to complementary sequences in the targeted mRNA and modify pre-mRNA splicing to correct the reading frame of a mutated transcript so that gene expression is restored. The rapid steady advances made in this field suggest that it is likely that AO-induced exon skipping will be the first gene therapy for DMD to reach the clinic. Indeed two different chemistries of AO continue to show encouraging results in clinical trials targeted at skipping exon 51 of the *DMD* gene, skipping of which would have the potential to treat 13% of DMD patients. However, the different deletions that cause DMD would require skipping of different exons, and the clinical work-up of other Aos. A major UK consortium is currently developing a peptide-conjugated AO for the targeted skipping of exon 53 for use in the next clinical trial. This AO would have the potential to treat 8% of DMD patients. Detailed comparative analysis of an array of overlapping conjugated and naked Aos has been performed in DMD patient cells and the choice of AO for the next clinical trial will be presented.

Poster 11

Optimal dystrophin mini-construct for gene delivery to skeletal muscle

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Dystrophin is the largest known human gene and contains 79 exons, covering 2.5 megabases of genomic DNA. The dystrophin cDNA sequence is 14 kb, encoding a 427 Kda protein. The large size of the dystrophin cDNA makes gene transfer strategies to treat DMD by transferring 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 to determine which parts of dystrophin can be removed without resulting in a functional loss. A number of different dystrophin truncated forms encoding the various components of the dystrophin molecule have been designed and cloned using overlap extension PCR. The constructs will be assessed using 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 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 closer to a rapid testing system and a full systemic pre-clinical trial in *mdx* mice.

Poster 12

Neurobehavioural disorders in Duchenne Muscular Dystrophy

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Objective Intelligence is one SD below average in Duchenne muscular dystrophy (DMD), correlating with brain dystrophin isoform expression. Variable neurobehavioural disorders are also seen in (DMD), although information on their prevalence and severity is incomplete. Dystrophin mutations affecting the central part of the gene and 3' disrupt brain dystrophin expression with more severe CNS phenotypes. We aimed to assess the DMD neurobehavioural profile and genotype relationship.

Methods DMD neuromuscular outpatient attendees, with standard-of-care treatment, were randomly recruited. Parents and teachers completed the Social-Communication Disorders Checklist (SCDC) and Strengths-Difficulties Questionnaire (SDQ). Scores were compared to normative data (SCDC mean 2.9, SD 4; SDQ mean 9.1, SD 6) and grouped according to genotype.
Results We recruited 78 boys, mean age of nine years. Thirteen had severe learning disability. Mean SCDC parental score was 9.1 (SD 6.7, $p<0.001$). Thirty-eight boys had scores predictive of autistic traits: 10/25 boys with mutations upstream of exon 30 had abnormal SCDC scores, 25/45 with mutations between exons 30-62, and ¾ with mutations downstream of exon 63. Boys with mutations known to affect all brain dystrophin isoforms had a higher SCDC mean score. Parent/teacher SCDC score correlation was good ($r=0.59$, $p<0.01$). Parent-completed SDQ mean score was 13.2 (SD 6.3, $p<0.05$). Pro-social, conduct and emotional domains were abnormal.
Conclusions In our screening, autistic traits and neurobehavioural disorders emerged as important facets in DMD. Whilst further exploring the yet poorly understood role of dystrophin in the CNS, more in depth assessments are required to delineate the cognitive-behavioural phenotype and genotype in DMD and to provide targeted support.

Poster 13
Assessing Viral Rescue Therapies for Duchenne Muscular Dystrophy
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Rescue therapies targeting post-transcriptional processing of the dystrophin gene – such as exon skipping and premature stop codon read-through – are in clinical trials, but unfortunately only bring hope for Duchenne Muscular Dystrophy (DMD) patients with specific mutations. Viral vector-mediated delivery of smaller variants of the dystrophin gene may restore dystrophin expression, as shown in animal models. This would alleviate symptoms in most DMD patients. Currently there is a bias towards successful dystrophin restoration in skeletal muscle with less encouraging results for cardiac tissue.
DMD patient fibroblast-derived induced pluripotent stem cells may be used as a model for testing various dystrophin restoration therapies *in vitro*. Furthermore, these cells can be differentiated into cardiomyocytes, offering a novel platform for confirming efficiency in cardiac restoration of dystrophin. There are various outcome measures that may be used to determine this.
We propose a new potential outcome measure for determining dystrophin restoration *in vitro*, based on the hypertrophic nature of dystrophic heart tissue. Two-dimensional measurements of primary embryonic *mdx* cardiomyocytes have shown a significant increase in size compared to C57.BL/10 control cells when removing serum from growth medium. Further measurements to confirm these results are needed, but may form a novel basis for testing cardiac tissue dystrophin restoration with viral and other rescue therapies.

Poster 14
Roles of a3-integrin in development of the neuromuscular system
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Integrins are a class of cell surface adhesion molecules responsible for mediating various cell functions, including motility, regulation of cell shape, differentiation and development. A3-integrin is a laminin receptor that has been implicated in various processes, including neuronal migration and working memory in the brain. In addition, it is present at the pre-synaptic side of the neuromuscular junction (NMJ), where its function is unknown; however, it has been demonstrated to bind laminins in the synaptic cleft, and voltage-gated calcium channels in the membrane, which are essential for

neurotransmitter release. However, the function of α3-integrin in development of the neuromuscular system *in vivo* has not been reported.
Here we investigate innervations and skeletal muscle development in α3-integrin knockout mice (α3-KO). We show that a3-integrin is expressed at the costameres; we report that it is not essential for the innervation and clustering of the acetylcholine receptors in the NMJs of developing embryos. Histochemical observations also revealed no obvious defects in the formation of intercostal and limb muscles, in contrast to previous reports showing a role for α3-integrin in myoblast differentiation *in vitro*. These data suggest that other integrin subunits compensate for α3-integrin's function in developing muscle; likely candidates are the other laminin-binding integrins α6 and α7 (both in conjunction with β1-integrin). Further studies aim to evaluate the roles of α3-integrin in the development of the NMJ.

Poster 15
Flow Cytometry in the Assessment of Functional Alpha-Dystroglycan Glycosylation in Dystroglycanopathy Patient Fibroblasts
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Alpha-dystroglycan (ADG) is a peripheral membrane protein that is an integral component of the dystrophin-glycoprotein complex. In an inherited subset of muscular dystrophies known as secondary dystroglycanopathies, ADG has reduced levels of glycosylation which results in lower laminin binding, as detected by the anti-dystroglycan antibody IIH6 and laminin blot overlay studies. The IIH6 antibody can serve as a biomarker for these disorders as it binds a specific glycan epitope on ADG involved in laminin binding. Immunocytochemistry is one of the most widely used methods to detect the level of ADG glycosylation in muscle. However while the interpretation of presence or absence of the epitope on muscle fibres is straightforward, the assessment of a mild defect can be challenging. In this study, flow cytometry was used to assess the level of IIH6-reactive glycans in dystroglycanopathy patient fibroblasts as well as control fibroblasts. This was done to see if the amount of IIH6 positive glycans could be accurately determined by this method to aid diagnosis. A total of 16 dystroglycanopathy patient fibroblasts have been analysed by this method: six with an unknown gene defect but a secondary dystroglycanopathy phenotype, four with FKRP mutations, 3 with POMGnT1 mutations, 2 with POMT1 mutations, and 2 with POMT2 mutations. Control fibroblasts as well as patient fibroblasts have clearly detectable levels of glycosylated dystroglycan. This test could complement existing diagnostic assays as it is both simple to perform and reproducible.

Poster 16
Ocular findings in Duchenne Muscular Dystrophy – an observational case series
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Background Electroretinogram (ERG) abnormalities are found in Duchenne Muscular Dystrophy (DMD). Some isoforms of dystrophin are highly expressed in retina. We aimed to expand on genotype associated retinal physiology.
Subjects and Methods 15 DMD boys attending the neuromuscular outpatient department and Glucocorticoid treated, mean age 11 years, (range 8-15 years) underwent fundoscopy, visual acuity, colour vision (Ishihara Plates), intra-ocular pressure measures (IOP) and full field flash ERGs to a range of scotopic and photopic flash strengths. Six patients had mutations involving exons 3-13, seven patients exons 44-57 and two patients exon 70.
Results Visual acuities were normal and no lens opacities were remarked. 2/15 boys had raised IOP, 1/15 showed R/G colour deficiency. All patients with mutations involving exons 44-57, one patient with mutation at exon 8-13 and one at exon 70 showed 'negative' scotopic ERGs with reduced b-wave amplitudes. The 15Hz scotopic flicker lacked a slow rod pathway response in these cases. The scotopic oscillatory potential, OP2, was reduced in all patients with mutations of exons

44-57, and in one patient exon 8-13. In contrast the photopic OP2 was preserved in these patients, but reduced in others with deletions upstream of exon 30. Photopic ERG b-waves were reduced variably across the patient group.
Conclusions Our study highlights a strong association of ERGs abnormalities with mutations downstream of exon 30, but the scotopic 'negative' ERG phenotype does not associate uniquely with specific retinal isoforms of dystrophin. Other ERG characteristics provide insight into the complex mechanisms of retinal signal disruption in DMD. Routine monitoring of IOP is recommended.

Poster 17
Identifying genomic pre-clinical biomarkers for diagnostics and therapeutics of Duchenne Muscular Dystrophy
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Duchenne muscular Dystrophy (DMD) is an inherited disease caused by mutations in the dystrophin gene that disrupt the open reading frame. Despite having mutations in a common gene individual patients respond differently to steroid treatment, suggesting that other factors such as modifying genes play a role in determining the phenotype.
In order to find candidate biomarkers for DMD we are performing whole exome sequencing in DMD patients with varying severity defined by the age of loss of ambulation. Five DNA samples from patients with early loss of ambulation and 5 DNA samples from patients with late loss of ambulation were successfully sequenced and produced an average of 2.8 Gb sequencing data with mean coverage of the sequenced regions of x50.
Our preliminary analysis showed that in patients with early loss of ambulation the identified variants affect genes involved in extracellular matrix organization such as FBN3, FBN2 , COL5A3, COL15A1, NRAP, LAMA1 and genes involved in inflammation response such as NOD1 and ITGA1 None of these groups of genes showed variants that were specific for the late loss of ambulation. Further validation of the identified SNVs by Sanger sequencing and also by genotyping in a larger DMD cohort will be carried out in order to confirm their possible role as modifying genes in DMD.

Animal Models of Neuromuscular Disease

Poster 18
Beta-blocker/ACE-inhibitor combination treatment in mdx mice
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Cardiomyopathy is the major cause of mortality in Duchenne Muscular Dystrophy (DMD) patients. We wished to explore the advantages of early treatment with a combination of ß-blocker and ACE-inhibitor, over treatment with each drug in isolation. We were particularly interested to learn if these treatments would have an impact on the pronounced right ventricular pathology seen in mdx. Male mdx mice were treated with metoprolol and/or captopril for 8 weeks from 16 weeks of age (prior to development of overt cardiomyopathic features) and assessed by MRI and cardiac conductance catheter at 24 weeks of age. Combination treatment produced a reduction in left ventricular mass and increased ejection fraction. However, 24 week old mdx mouse hearts are not hypertrophic and have a normal ejection fraction at baseline. Metoprolol and combination treatment both resulted in a normalization of left ventricular contractility (as measured by ESPVR) however none of the treatments were effective in preventing right ventricular dysfunction or global cardiac histopathology. Overall, there appeared to be little added benefit of administering captopril with metoprolol in the mouse model of DMD.

Poster 19
Structural brain defects associated with a knock-down in Fukutin related protein in the mouse
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Mutations in fukutin-related protein (FKRP) are associated with the hypoglycosylation of α-dystroglycan, which effectively disrupts the linkage between the dystrophin-associated glycoprotein complex and the extracellular matrix. These mutations are responsible for a clinically heterogenous group of muscular dystrophies, which vary in severity from severe congenital muscular dystrophies, with substantial brain and eye involvement, to relatively mild adult-onset limb girdle muscular dystrophies. To investigate the pathogenesis of these diseases, we have developed a mouse model, which has approximately 80% reduction in levels of FKRP. The brain of this mouse displays a marked disturbance in the deposition of laminin α-chains including α1, α2, α4, and α5 at the pial basement membrane, together with a diffuse pattern of laminin deposition below the pial surface (1). In view of the previously reported spectrum of structural defects, ranging from complete lissencephaly in patients with Walker-Warburg syndrome to isolated cerebellar involvement (2) we now present a detailed characterisation and localisation of the neuropathologic findings in the newborn FKRP knock-down mouse.

1. Ackroyd,M.R., Whitmore,C., Prior,S., Kaluarachchi,M., Nikolic,M., Mayer,U., Muntoni,F., Brown,S.C. (2011) Fukutin-related protein alters the deposition of laminin in the eye and brain. *J. Neurosci.*, 31, 12927-12935.
2. Clement,E., Mercuri,E., Godfrey,C., Smith,J., Robb,S., Kinali,M., Straub,V., Bushby,K., Manzur,A., Talim,B., *et al.* (2008) Brain involvement in muscular dystrophies with defective dystroglycan glycosylation. *Ann. Neurol.*, 64, 573-582.

Poster 20

Targeting the endogenous stress response in a mouse model of SBMA

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Spinal and bulbar muscular atrophy (SBMA) is an inherited, X-linked, adult onset motor neuron disorder caused by a polyglutamine (CAG) repeat expansion in exon 1 of the androgen receptor (AR) gene. Disease manifestation is androgen dependant so that clinical presentation is predominantly restricted to males and is characterised by weakness, atrophy and fasciculations of facial, bulbar and proximal limb muscles, leading to significant disability.

We are investigating 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). These mice develop late onset lower motoneuron degeneration with accompanying progressive neuromuscular phenotype, which recapitulates both pathologic and phenotypic characteristics of the human disease.

We are undertaking a preclinical trial of arimoclomol, a pharmacological co-inducer of the endogenous heat shock response (HSR). Arimoclomol has previously been shown to significantly improve disease phenotype in the SOD1 mouse model of Amyotrophic Lateral Sclerosis (ALS), a rapidly progressing motoneuron disease (Kieran *et al*, 2004; Kalmar *et al*, 2009) and is currently in phase II/III randomized, placebo-controlled clinical trials for ALS. *In vivo* assessment of neuromuscular function at designated end stage (18 months) in the AR100 mice suggests a beneficial effect when treatment is initiated at symptom onset (12 months). We are currently investigating the effects of a presymptomatic treatment regime, an approach that is relevant to the management of SBMA patients, as genetic identification of at-risk individuals is possible.

Poster 21

Basement membrane deposition in the skeletal muscle of the FKRPknock-down mouse.

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Mutations in fukutin-related protein (FKRP) are associated with a clinically heterogenous group of muscular dystrophies, which vary in severity from severe congenital muscular dystrophies, with structural brain and eye involvement, to relatively mild adult-onset limb girdle muscular dystrophies. Central to the pathogenesis of these disorders is the hypoglycosylation of alpha dystroglycan, and defects in basement membrane deposition. We previously generated a mouse model, which has an approximately 80% reduction in levels of FKRP. The newborn brain of this mouse displays a marked reduction in alpha dystroglycan glycosylation and a disturbance in the deposition laminin at the pial basement membrane. In skeletal muscle there is a reduction in both alpha dystroglycan and laminin alpha 2 immunolabelling by the time of birth in the FKRP knock-down compared to controls (1). There is in addition an associated reduction in muscle mass of the FKRP knock-down, the origin of which is unclear. Here we present our preliminary findings with regard to the expression of alpha dystroglycan and its associated ligands laminin alpha 2 and perlecan during muscle development and at birth in the FKRP knock-down relative to wild type controls. Overall our data shows that alpha dystroglycan and its extracellular matrix ligands laminin alpha 2 and perlecan immunolabelling clearly delineate the basement membrane at E15.5 in the mouse. This is a period when secondary myotubes align along the surface of the primaries suggesting that a reduction in the glycosylation of alpha dystroglycan may influence secondary myogenesis.

1. Ackroyd,M.R., Whitmore,C., Prior,S., Kaluarachchi,M., Nikolic,M., Mayer,U., Muntoni,F., Brown,S.C. (2011) Fukutin-related protein alters the deposition of laminin in the eye and brain. *J. Neurosci.*, 31, 12927-12935.

Poster 22

Chemical inhibitor treatment of a zebrafish model of muscular dystrophy

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Dystrophin forms part of the DGC (dystrophin-associated glycoprotein complex) at the membrane of muscle cells. The DGC links the actin cytoskeleton to the extracellular matrix, and this linkage protects the cell from damage during muscle contractions. Loss of dystrophin results in the loss of the other DGC components from the membrane, leading to the muscle wasting seen in Duchenne muscular dystrophy. At the centre of the DGC is the transmembrane glycoprotein, dystroglycan, and its restoration to the membrane can restore other components of the DGC. However, this has not yet been shown to ameliorate the dystrophic phenotype, possibly due to failure to prevent the continued internalisation/degradation of dystroglycan. The proteasome pathway has been implicated in the degradation of DGC components. Tyrosine phosphorylation of dystroglycan has also been shown to play a role in the loss of dystroglycan from the plasma membrane, promoting its internalisation. We have developed an assay to screen chemical inhibitors of pathways known to contribute to dystroglycan recycling/degradation to assess the extent to which muscle attachments can be strengthened in the dystrophic mutant zebrafish (sapje). The proteasomal inhibitor MG132 was found to be effective in reducing the muscle damage in sapje, with an EC50 of 0.4μM. These inhibitor studies may provide an insight into the molecular mechanisms that lead to the loss of dystroglycan and associated proteins from the membrane in dystrophic muscles, which may have future therapeutic implications.

Poster 23

Investigating New Mutant Models of MND

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Mutations in superoxide dismutase 1 (SOD1), a cytoplasmic and mitochondrial enzyme, account for approximately 10% of familial Motor Neuron Disease (MND) cases. Mutant SOD1 has been associated with abnormal mitochondrial and neuronal function *in vitro*. However the majority of experimental data regarding the pathological activity of mutant SOD1 has come from transgenic mouse models overexpressing the mutant human protein. We have a mutant mouse created by the ENU mutagenesis project at MRC Harwell, with a single point mutation (D83G) in endogenous mouse Sod1, which is analogous to a pathogenic mutation identified in several families. The mutant protein is expressed in these mice at physiological levels and is not confounded by excessive overexpression. We have observed motor neuron degeneration in these mice and so in order to investigate the underlying cellular pathology, primary embryonic motor neurons were isolated from Sod1 D83G mice on embryonic day 13.5 and cultured and neuronal development and mitochondrial function were examined. After 18 hours *in vitro*, we observed significant differences in neurite outgrowth, in particular of axon length, in heterozygotes and homozygotes compared to wildtype motor neurons. Homozygote motor neurons did not survive to 7 days *in vitro*, so mitochondrial membrane potential could only be determined in heterozygote and wildtype motor neurons. The results revealed a significant hyperpolarisation of the mitochondrial membrane potential in heterozygote motor neurons. These results suggest that the D83G mutation in mouse Sod1 disrupts neuronal development and mitochondrial function, which may contribute to motor neuron dysfunction and degeneration in these mice.

Poster 24

Investigating the effects of DAO transgenes on the SOD1^{G93A} mouse model of Amyotrophic Lateral Sclerosis (ALS)

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Amyotrophic Lateral Sclerosis (ALS) is a lethal motor neuron disease, characterized by selective and progressive degeneration of both upper and lower motor neurons. 20% of the hereditary cases are associated with mutations in copper/zinc superoxide dismutase-1 (SOD1) protein and the remaining familial cases arise from mutations in other genes, including the *DAO* gene, which encodes D-amino acid oxidase protein (DAO), a peroxisomal flavin adenine dinucleotide (FAD)-dependent oxidase protein.

We aimed to investigate the role of DAO in ALS pathology, using a series of survival studies involving transgenic mice over-expressing mutant or wild-type DAO. In particular we were interested in assessing the impact of the DAO transgene on the disease development in the SOD-1^{G93A} mouse model. We mated SOD1^{G93A} and DAO^{R199W} heterozygotes to generate 4 genotypes (SOD-1^{G93A} + DAO^{R199W}, SOD-1^{G93A}, DAO^{R199W}, wild-type) for both sexes. The development of ALS related disability was assessed by means of neurological scoring system for both hind legs twice weekly for each mouse in conjunction with assessment of body weight. Neurological scores were assigned using a scale of 0-4, established by the ALSTDI, where 0 was the full extension of hind legs and 4 indicated the inability to right itself within 20 seconds.

Data collected showed that while the presence of the mutant DAO^{R199W} transgene did not have any significant effect on survival, mice bearing the DAO transgene displayed a lower body weight in comparison to their non-transgenic littermates. The second survival study (mating SOD1^{G93A} and over-expressing DAO wild type heterozygotes) is currently underway.

Poster 25

Validation of Novel Secondary Dystroglycanopathy Genes using Biochemical, Cellular and Zebrafish studies

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Mutations in several known genes such as LARGE, POMT1, POMT2, POMGnT1, FKRP, FKTN, DPM2 and DPM3 are capable of causing glycosylation defects in alpha-dystroglycan (ADG), an integral component of the dystrophin glycoprotein complex. This contributes to the pathogenesis of an inherited subset of muscular dystrophies known as the dystroglycanopathies at least in part by reducing laminin binding. There are undoubtedly many more genes to be discovered which contribute to this defective glycosylation as only approximately 50% of dystroglycapathy patients can be diagnosed as having a defect in any of these known genes. The results of the UK10K project led to the identification of two patients with compound heterozygous mutations in two novel genes, both of which are likely to be involved in glycan processing. We will present the results of zebrafish morpholino knock downs of these two genes as well as protein studies including transfection, RT-PCR to determine tissue and age related distribution, western blotting, and flow cytometry to determine the extent of the reduction of ADG glycosylation in patients' cell lines. Thus far these studies support the hypothesis that the mutations are causative.

Poster 26

Poloxomer 188 has a deleterious effect on skeletal muscle function in the *mdx* mouse

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Poloxomer 188 (P188) is a tri-block copolymer that has been proposed as a potential treatment for cardiomyopathy in Duchenne Muscular Dystrophy (DMD). Despite the reported beneficial effects of P188 on dystrophic cardiac muscle function, the effects of P188 on dystrophic skeletal muscle function are relatively unknown. Here we report that P188 impairs skeletal muscle function, questioning its suitability as a therapeutic for DMD. *Mdx* mice were injected intraperitoneally with 460mg/kg or 30mg/kg P188 dissolved in saline, or saline alone. The effect of single-dose and 2-week daily treatment was assessed using an *in vivo* exercise test on the *Tibialis Anterior* (TA) muscle *in situ*. The test comprised a warm up, measurement of the force-frequency relationship and a series of eccentric contractions with a 10% stretch. P188 treatment at either dose did not significantly affect the force-frequency relationship or maximum isometric specific force produced by the TA muscle. However, after 2 weeks of P188 treatment at either 30 or 460mg/kg/day the drop in maximum force produced following eccentric contractions was significantly greater than that seen in saline treated control mice (P=0.029, Repeated Measures ANOVA). There was no difference in force drop during eccentric exercise between the two doses. A trend for a greater drop in force was seen after a single dose 30mg/kg dose of P188 however this was not statistically significant. High dose P188 also increased the bleeding time. In conclusion P188 treatment increases susceptibility to contraction-induced injury in dystrophic skeletal muscle which may outweigh its potential cardiac therapeutic properties.

Poster 27

Does resting laryngoscopy accurately predict severity of muscle histopathology in equine recurrent laryngeal neuropathy?

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Introduction: Recurrent laryngeal neuropathy (RLN) is a common equine distal axonopathy associated with neurogenic atrophy of the intrinsic laryngeal muscles (particularly on the left). Affected horses develop laryngeal paralysis and poor performance. Although the aetiology is unknown, a genetic cause is suspected. We hypothesised that severity of laryngeal dysfunction determined by videolaryngoscopy, would be correlated with severity of laryngeal muscle histopathology. If so the technique could be used to identify unaffected animals and to grade disease severity for genetic studies and quantitative trait analysis.

Methods: Laryngeal function was graded from videolaryngoscopy recordings in 29 horses. Subsequently, intrinsic laryngeal muscles were collected and analysed for fibre types, type grouping, minimum fibre diameter, % collagen and % fat.
Results: The left cricoarytenoideus dorsalis muscle had significantly more collagen and fat than the right in horses with complete laryngeal paralysis. Discriminant analysis revealed significant overlap in muscle pathology between horses with different degrees of laryngeal function, with the exception of horses with complete left sided abductor paralysis. Fibre type grouping was observed frequently, particularly on the left side.
Conclusions and potential relevance: Laryngeal function – as determined by resting laryngoscopy – does not correlate with severity of histopathology; horses with normal laryngeal function may have subclinical RLN. Resting laryngoscopy may lead to inaccurate phenotyping in genome wide association studies and quantitative trait analyses. This study provides valuable information that will allow muscle histopathology to be used as an outcome measure in the future investigation of novel treatments for RLN.

Poster 28
Assessing the therapeutic potential of *LARGE* in a mouse model for the limb girdle muscular dystrophies
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Mutations in 8 genes, including 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 Walker Warburg Syndrome. We have now generated a mouse with a knock-down in Fkrp expression in the skeletal muscle, but not the central nervous system ($FKRP_{MD}$). Analysis of skeletal muscle from this mouse demonstrates hypoglycosylation of α-dystroglycan and clear muscle pathology by 12 weeks of age. Previous work has shown that LARGE overexpression induces hyperglycoyslation of α-dystroglycan in wildtype cells and additionally cells from dystroglycanopathy patients with various primary gene defects, suggesting LARGE could be an important therapeutic approach in these disorders. To test this strategy for FKRP associated disorders, we have crossed our $FKRP_{MD}$ mouse line with a second line transgenic mouse line overexpressing LARGE. Here we present our histological and physiological evaluation of these mice ($FKRP_{MD}$*LV5).

Poster 29
Investigating Basement Membranes in FKRP and Fukutin Deficient Zebrafish
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Fukutin-related protein (FKRP) and fukutin play a role in the glycosylation of α-dystroglycan, a key receptor for basement membrane proteins. Aberrant α-dystroglycan glycosylation leads to defects in the assembly of the basement membrane and it is involved in the pathogenesis of a broad spectrum of disorders, ranging from limb-girdle muscular dystrophy to muscle eye brain disease and Walker-Warburg syndrome. Here we investigate the structural changes in muscle, eye and notochord of 3dpf zebrafish larvae, with particular emphasis on the basement membrane. Antisense oligonucleotide morpholinos were used to knock down FKRP and fukutin in wild type zebrafish. The morphants had abnormal muscle fibres, disrupted vertical myosepta and sarcolemma. The area of the notochord was observed to be smaller in all morphants when compared to controls. Electron microscopy revealed disturbances in all three layers that form the peri-notochord sheath including the basement membrane. Toluidine blue staining showed disorganised retinal layering in both

morphants. Dysplasia of the lens was observed in most fukutin morphants and two FKRP morphants with a severe phenotype. Transmission electron microscopy studies revealed a homogenous perturbation across the inner limiting membranes of both morphants which may account for the lens dysplasia. The rod and cones in the photoreceptor cell layer were found in lower density in both morphants with the least density in fukutin knock-downs which may be as a result of a disrupted external limiting membrane. We therefore conclude that FKRP and fukutin are essential for the integrity of membranes in the eye, muscle and notochord of developing zebrafish larvae.

Muscle Satellite Cells

Poster 30
Labelling satellite cells on human muscle fibres

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Satellite cells, normally quiescent underneath the myofibre basal lamina, are skeletal muscle stem cells responsible for postnatal muscle growth, repair and regeneration. Since their scarcity and small size have limited study on transverse muscle sections, techniques to isolate individual myofibres, bearing their attendant satellite cells, were developed. Studies on mouse myofibres have generated much information on satellite cells, but the limited availability and small size of human muscle biopsies have hampered equivalent studies of satellite cells on human myofibres. Here, we identified satellite cells on fragments of human and mouse myofibres, using a method applicable to small muscle biopsies.

Poster 31
Role of Ret in satellite cell myogenesis and Facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is the third commonest inherited myopathy, linked to deletion of tandem 3.3kb repeats (*D4Z4* units) in chromosome 4q35. There is an ORF within each D4Z4 repeat that encodes a double homeodomain protein – DUX4, and loss of a critical number of *D4Z4* units results in DUX4 expression. In FSHD, there is evidence that satellite cell function is directly affected, and DUX4 and DUX4c message and protein have been found in FSHD patient myoblasts. Expression of DUX4 in mouse satellite cells inhibits myogenic differentiation and is toxic. To unravel how DUX4 exerts its deleterious effects, we have performed a microarray screen on satellite cells following infection with retroviruses encoding DUX4. Of the significant transcriptional changes elicited by DUX4, one gene of particular interest was *Ret* (rearranged during transfection): a receptor tyrosine kinase activated by the Glial Derived Neurotrophic Factor (GDNF) family ligands. The up-regulation of Ret by DUX4 suggests a role in FSHD pathology. Furthermore, Ret is required for myogenesis in certain facial muscles in Zebrafish. However, Ret has not yet been examined with regards to satellite cell function. We have found Ret is dynamically regulated in murine satellite cells during myogenic progression. Therefore, we are investigating the function of Ret in satellite cell function in health and disease using retroviral-mediated constitutive expression and siRNA-mediated knockdown, and will present preliminary data that Ret/Ret mutants affect myogenic differentiation.

Poster 32
Polycomb group genes and the regenerative process in the aged and pathological human muscle

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Skeletal muscle is able to regenerate throughout the life of individuals due to a resident population of stem cells called 'satellite cells'. These satellite cells are normally quiescent and located beneath the basal lamina of the muscle fibres, after muscle injury or in diseases the satellite cells become

activated and re−enter the cell cycle to provide repair cells as well as re−populating the stem cell population. In ageing and in muscle diseases there is degeneration of the skeletal muscle which is linked to changes in the ability of the satellite cells to repair the muscle leading to a progressive worsening of the pathology.
We have recently shown in mouse models that Bmi1, a member of the Polycomb group gene family (PcG), is expressed in both the quiescent and proliferating satellite cells. Moreover, we have demonstrated that Bmi1 deficiency leads to a reduction in the number and ability of the remaining satellite cells to contribute to regeneration.
Here, we have analysed the expression of selected PcG genes (BMI1, EZH2, EED1) and regulatory genes (JMJD3, MLL1) in normal muscle biopsies from adult individuals across the whole age range and in pathological human muscle biopsies. qRT-PCR on RNA extracted from the biopsies has been carried out to gain quantitative data and immunofluorescence including co-localisation with markers of quiescent and activated satellite cells have been performed. We show a decline in the expression of BMI1 in the aged muscle. Moreover, while the expression of BMI1 is either increased or kept constant in muscle biopsies showing regeneration without evidence of an underlying chronic myopathic process, its expression is dramatically reduced in chronic myopathic muscle conditions. These data raise the possibility that PcG genes play an essential role in controlling regeneration also in the human muscle.

Muscle Channelopathies and Myasthenia Gravis

Poster 33
Uncommon neurophysiological pattern in Lambert Eaton syndrome

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INTRODUCTION: Lambert Eaton syndrome is an uncommon myasthenic syndrome, generally correlated to small lung cancer carcinoma. Electrophysiologic evaluation is mandatory , generally showing a decrement of C-MAPs (compound action potentials) a t slow frequency repetitive nerve stimulation (RNS) and increment of C-MAP greater then 40% (calculated by(100%(highest amplitude-I amplitude)/initial amplitude)).
CASE REPORT: We present a 66 year old man patient who complained of astania, mild proximal weakness, diplopia looking extremely right and dizziness. Low and high-frequency RNS were performed , showing a 30-40% C-MAP decrement at low RNS (3Hz) and none C-MAP variation at high-frequency RNS (30Hz). Antibodies against acetylcholine receptor were negative and the patient was poorly responder to pyridostigmine. Thethoracic CT scan performed showed a big mediastinic-pulmonary mass, which showed to be a small cell lung carcinoma at hystolocgical examination. A performed cerebral MRI with gadolinium was normal. D We registered very high title of IgG antibodies against presynaptic voltage-gated calcium channel (VGCC) .
CONCLUSION: This case show an atypical neurophysiological high frequency RNS pattern in Lambert Eaton syndrome.

Poster 34
Muscle degeneration in ion channel dysfunction

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Mutations in the skeletal muscle voltage gated sodium channel (NaV1.4) or calcium channel (CaV1.1) lead to an array of degenerative muscle diseases. While the direct effects of mutations are known, the reason for longer term degeneration is not. This project therefore aims to clarify the pathophysiology resulting from such mutations. To this end, two models are being developed.
The first and main model is myotubes, produced from fibroblasts that are donated by patients and control subjects by tranfection with an electron deleted adenovirus which was modified to deliver transcription factor MyoD to cells, causing them to transform into myoblasts. These are cultured for fluorescence lifetime imaging, oxygen consumption measurements and immunofluorescence assays. Initial results from a control line of fibroblasts have established that these cells do indeed form

myotubes upon viral treatment. These myotubes respond both to caffeine and to high potassium, and are thus likely to be a viable model for further experiments on patient cells.
The second model is mutant and control mice. Changes in extensor digitorum longus (EDL) and tibialis anterior (TA) tension and fatigue are measured. Initial results suggest that wild type mice have stronger EDL and TA than mice carrying a mutation in their skeletal Sodium channel.

Poster 35
The spectrum of mutations that underlie the *DOK7* neuromuscular junction synaptopathy, and the patient response to treatment
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Congenital myasthenic syndromes are a group of inherited disorders that affect synaptic transmission at the neuromuscular junction and result in fatiguable muscle weakness. A sub-group of patients, approximately 20% of CMS kinships, have recessively inherited limb-girdle weakness caused by mutations in *DOK7*. *DOK7* encodes Dok-7, an adaptor protein that is expressed in skeletal muscle and heart and that is essential for the development and maintenance of the neuromuscular junction. In 65 kinships we have identified 34 different mutations and also note another 27 variants that are likely to be non-pathogenic. A C-terminal frameshift duplication 1124-1127dupTGCC is commonly found in at least one allele, 24/65 kinships, with the other allele often being located in the N-terminal half of the protein. We have analysed the effect of this common frameshift mutation and 11 missense variants located in the N-terminal domains of Dok-7 on clustering of AchR *in vitro*. In addition we identify synonomous coding variants within the N-terminal exons that cause defective splicing. Previously, reported cases of *DOK7* CMS have all had at least one allele harbouring a mutation in the C-terminal exon 7. Functional studies have enabled us to identify and characterise patients who are homozygous or compound heterozygotes for mutations within the N-terminal domains. The patients show a dramatic response to treatment with beta2-adrenergic receptor agonists ephedrine or salbutamol.

Poster 36
Progesterone reduces and shifts the voltage dependence of the skeletal muscle chloride conductance
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The skeletal muscle chloride channel, ClC-1, regulates muscle fibre excitability by resisting deviations from the resting potential and by determining the cell's input resistance at rest. Relatively little is known about the signalling pathways that control ClC-1 expression levels, nor about the pathomechanisms underlying phenotypic variability in low chloride conductance myotonia (Myotonia Congenita). Our group previously showed that progesterone, but not oestrogen shifts the voltage dependence of human ClC1 heterologously expressed in Xenopus oocytes, and that a homone-induced voltage shift can exacerbate the effect of a Myotonia Congentia mutation. However, signalling pathways in amphibian oocytes are likely to be different from those in mammalian muscle. Here we present data from wildtype mouse flexor digitorum brevis showing that progesterone induces a right-shift of voltage dependence and reduction in amplitude of the endogenous skeletal muscle chloride current. Oestrogen exerts a qualitatively similar but less pronounced effect. This work provides further support for the idea that sex hormones contribute to the exacerbation of Myotonia Congenita that can occur during pregnancy.

Poster 37
A retrospective clinical study of the treatment of slow-channel congenital myasthenic syndrome

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Background and objectives Slow-channel congenital myasthenic syndrome (CMS) is a rare subtype of CMS caused by dominant "gain of function" mutations in the acetylcholine receptor (AchR). Clinically, the cervical and forearm extensor muscles seem to be preferentially weaker; and conventional treatment with anticholinesterases fails to improve symptoms. In contrast, open channel blockers such as fluoxetine and quinidine have been shown to be of benefit. The objectives of our study were to provide further insight into the clinical features of slow-channel CMS and evaluate response to recommended therapy.
Method and Patients We carried out a retrospective clinical follow up study of 15 slow-channel CMS patients referred to the Munich CMS Centre. Detailed clinical data were collected by clinicians involved in the care of each patient, with a particular focus on response and tolerability to recommended therapy.
Results Our results support previous reported findings in terms of clinical features as well as the poor response to pyridostigmine. We were interested to note that although treatment with fluoxetine was beneficial, a number of our patients suffered significant adverse effects that hindered optimum dose titration or led to treatment cessation. Patients receiving quinidine seem to tolerate this treatment better.
Conclusion Slow channel CMS are a rare category of CMS with distinct clinical and neurophysiological features. Establishing the underlying genetic diagnosis is essential in selecting the correct treatment. In contrast to other published series, our study suggests that fluoxetine can be associated with significant side effects thus reducing treatment effectiveness.

Poster 38

Episodic Muscle and Brain channels: Analysis of the *PRRT2* gene and screening of a muscle channel panel

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Abstract: A number of genetic causes have been identified that cause episodic movement disorders. These are exhaustive to screen and often difficult with the presence of deletions and clear heterogeneity. We screened the proline-rich transmembrane protein (*PRRT2*) gene in a large series of episodic neurological disorders (such as paroxysmal dyskinesia (PKD), episodic ataxia (EA) and hemiplegic migraine (HM) and designed muscle and episodic neurological disorder screening panels to enable comprehensive genetic testing.
Methods: 1. The PRRT2 gene was sequenced in 58 family probands/sporadic individuals with PKD/IC, 182 with EA, 128 with HM and 475UK and 96 Asian controls. We plan to further investigate these mutations in cell models in collaboration with Prof Ptacek's group. 2. Design of a muscle channel and an episodic brain channel screening panel to identify point mutations and deletions/insertions.

Results and Conclusions**:** *PRRT2* genetic mutations were identified in 28 out of 58 PKD/IC (48%), 1/182 EA and 1/128 individuals with HM. A number of loss-of-function and coding missense mutations were identified, the commonest mutation found was the p.R217Pfs*8 insertion. Males were more frequently affected than females (ratio 52:32). There was a high proportion of *PRRT2* mutations found in families and sporadic cases with PKD associated with migraine or HM (10 out of 28). One family had EA with HM and another large family with typical HM alone. The muscle channel 45uglier on the channels associated with myotonia and designed to pick up structural changes and the brain channel panel consisted of the genes associated with EA, including the *PRRT2* gene. The *PRRT2* gene is associated with the frequent occurrence of migraine and HM with PKD/IC, the association of mutations with EA and HM and with familial HM alone. We expect the muscle and brain channels to expand the phenotype of these disorders further.

Poster 39

Prevalence study of skeletal muscle channelopathies in England

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Introduction: The non-dystrophic myotonias (NDM) and periodic paralyses (PP) are a group of skeletal muscle disorders caused by mutations in genes encoding ion channels. To date, very few studies have systematically evaluated the prevalence of these disorders, and most of them predate genetic diagnosis.
Objective: To obtain prevalence data on skeletal muscle channelopathies and to evaluate the relative frequency of common mutations.
Methods: The study covered all patients with NDM or PP living in the UK that were referred to the UK national reference centre for assessment. Inclusion criteria were clinical and electrophysiological features of NDM or PP, and confirmed mutations in genes encoding ion channels. England was selected as the geographical area for prevalence analysis.
Results: From a total of 662 patients identified, 447 had NDM and 215 had PP. Of them, 590 were from England, giving a point prevalence of 1/100,000. Significant allelic heterogeneity was associated with NDM and Andersen-Tawil syndrome. However, a limited number of mutations were responsible for most cases.
Conclusion: We have analysed the largest series of patients with skeletal muscle channelopathies reported so far, and documented for the first time their overall prevalence. The spectrum of mutations was similar to that previously reported.

Poster 40

Episodic Ataxia: screening candidate genes and genetic analysis of families

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Background: Channelopathies are widespread in neurology, genetically hetrogeneous and the identification of disease genes instantly leads to therapeutic implications. Next generation DNA sequencing platforms have been widely available since 2008 and have led to a dramatic reduction in the cost and speed of DNA sequencing. This technology has been used to enable the development of whole genome and exome sequencing as both a research and diagnostic tool however, significant challenges remain with the application of both these techniques. The same technology can be used to enable rapid sequencing of smaller amplicons that is well suited to support current research and diagnostic sequencing requirements. This allows multiple genes to be sequenced rapidly and accurately at significantly reduced cost compared to Sanger sequencing. We have taken this approach to develop: 1. A screening panel for multiple genes that are known to cause episodic ataxia, 2. Linkage and exome sequencing of families and 3. In preparation for functional studies and collaborative bank of fibroblasts.

Methods: We designed our panel of genes for use with our Illumina MiSeq platform amplicons defining the coding regions of the known episodic ataxia genes. Small families were analysed using combination of linkage and exome sequencing. Fibroblasts were taken using an upper arm aseptic punch biopsy technique.
Results and conclusions: A total of 21,619 bp were sequenced per sample from 128 amplicons. The current technology allows 95 samples to be processed in the same run however this is likely to increase significantly in the future. A 2x 150bp paired-end run takes approximately 27hrs to complete on the MiSeq system. Exome sequencing on small families has yet to reveal new genes but we expect analysing families with similar phenotypes will yield important results.

Peripheral Nerve Disease

Poster 41
Proton sensitivity is differentially regulated in cell bodies and terminals of sensory DRG neurons *in vitro*

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Background and aims Protons are known to evoke a sustained activation of small, nociceptive sensory neurons, resulting in pain in humans. The molecular transduction mechanism of proton stimulation has been demonstrated to rely on distinct mechanisms, chiefly activation of TRPV1 or members of the family of acid-sensing ion channels (ASIC). Previous investigations have either focused on the soma or the terminals of cells in isolation and have provided contradictory evidence as to the contribution of each transduction mechanism. Here we analysed the spatial proton sensitivity of individual neurons. **Methods** We used a compartmentalised culture chamber in which the terminals, axons and soma can be fluidically separated with an isolating microgroove barrier. Dissociated rat E14 DRG neurons were seeded into one compartment and were encouraged to grow into adjacent compartments by using an NGF gradient. Ratiometric calcium imaging of the soma following application of protons were conducted at 7 DIV. **Results** Acid stimulation induced action potential propagation from terminals and axons. By using a pH dose response protocol, we showed that the sensory terminals have a greater sensitivity to protons than the cell soma. Approximately 80% of the responses induced by proton application to the terminals could be blocked by 100 µM amiloride. **Conclusions** We conclude that there is a significant, regional differential regulation of proton sensitivity within a sensory neuron in vitro, with ASICs contributing a significant transduction mechanism.

Poster 42
Genetic analysis of *FIG4* in patients with CMT

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Charcot-Marie-Tooth (CMT) disease is a genetically heterogeneous group of motor and sensory neuropathies associated with mutations in more than 30 genes. CMT type 4J is an autosomal recessive neuropathy caused by mutations in the lipid phosphatase *FIG4* gene. Few mutations have been reported so far and the majority of CMT4J patients have the compound heterozygous genotype $FIG4^{I41T/null}$ that was originally described in four families. So far, 16 cases have been described with *FIG4* mutations. CMT4J is clinically characterised by highly variable onset and severity, proximal as well as distal and asymmetric muscle weakness, a demyelinating neuropathy with electromyography demonstrating denervation in proximal and distal muscles and frequent progression to severe amyotrophy. In this study the 23 exons of the *FIG4* gene were sequenced by the use of standard-PCR and Sanger sequencing in patients with recessive CMT and patients with distal motor

neuropathy/neuronopathy that developed as a child or early adult. We preferentially selected cases where the disorder was asymmetrical.
All the patients were negative for mutations in the HSP22 and HSP27 genes. One patient was found to be a *FIG4* compound heterozygote carrying the previously reported I41T missense mutation and the protein truncation mutation p.K278YfsX5. This patient had early onset, severe demyelinating motor and sensory neuropathy with rapid onset of paralysis of his left arm over 6 months last year and no family history. This is the first case in the UK with CMT due to *a FIG4* mutation.

Poster 43

Hereditary sensory neuropathy type 1: correlation of severity and plasma atypical deoxy-sphyngoid bases

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Hereditary sensory neuropathy type 1 (HSN1) is an autosomal dominant peripheral neuropathy characterised by marked sensory loss, variable motor involvement and frequent sensory complications including amputations and ulcerations. Mutations in the L-serine palmitoyltransferase long chain subunit 1 (*SPTLC1*) and subunit 2 (*SPTLC2)* genes are responsible for this disorder. Mutant *SPTLC1* and *SPTLC2* are associated with the accumulation of two atypical deoxy-sphyngoid bases (DSBs) which produce a toxic effect in cultured sensory neurons. DSB levels are elevated in plasma of patients with HSN1 and transgenic mice. A recent trial of L-serine in transgenic mice expressing the mutant SPTLC1 showed reduction of the DSBs and an improvement in the phenotype. A pilot study of L-serine in 14 HSN1 patients similarly showed reduction of DSBs. L-serine is therefore an excellent candidate therapy for HSN1, however the lack of natural history studies and outcome measures are major barriers to a clinical trial. This cross-sectional study has been conducted to fully characterize the phenotype of HSN1 and to establish correlation of plasma DSBs with disease severity. We obtained detailed clinical data in a cohort of 25 HSN1 patients. Clinical impairment was assessed by the Charcot Marie Tooth Neuropathy score (CMTNS). Onset occurred in the second and third decade in the majority of patients. Males showed a more severe disease course compared to females. DSBs were significantly elevated in all patients and correlated with disease severity. DSB levels are a promising potential biomarker to be considered for a study of L-serine in HSN1.

Poster 44

Mutations of the kinesin family member 5A (*KIF5A)* gene in patients with pure or complex Charcot-Marie-Tooth type 2 (CMT2)

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Background KIF5A, the gene encoding the neuronal kinesin heavy chain (KHC) subunit of kinesin-1, was known as the responsible gene of spastic paraplegia type 10 (SPG10). In complex SPG10, axonal neuropathy has been recognized as the most frequently associated feature. KIF5A mutations can also rarely cause pure axonal neuropathy in a CMT2-like phenotype. This led to the hypothesis that SPG10 and CMT2 may be allelic diseases.
Objective To test the hypothesis that KIF5A can cause CMT2 and to investigate the phenotypic spectrum of KIF5A mutations.
Patients and methods We directly sequenced exons 1-12 of KIF5A encoding the motor domain of the protein in 401 CMT2 patients with predominant motor involvement in which mutations in known genes had been excluded.

Results Four different heterozygous missense mutations were identified in four unrelated patients: two novel mutations: c.G694A (D232N), c.T910A (C304S), and two mutations previously noted in SPG10: G839A (R280H); c.C838T (R280C). Three patients presented with CMT2 plus pyramidal signs. Although R280H presented with pure CMT2 in our case, it caused complex SPG10 plus axonal neuropathy in another family.
Conclusions This study is currently the largest series of CMT2 cases investigated for KIF5A mutations. The results confirmed KIF5A mutations can cause CMT2. We also broadened the phenotypic spectrum of *KIF5A* mutations; while the most common phenotype is spastic paraplegia plus axonal neuropathy, CMT2 can be the main manifestation particularly in patients with predominant motor neuropathy and pyramidal signs.

Poster 45
Variation in the disability in males of similar age with CMT1X
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Objective: To investigate the degree of clinical variability in males with Charcot–Marie–Tooth disease type 1X (CMT1X). **Background:** CMT1X is caused by mutations in the gap junction beta 1 (*GJB1*) gene, located on the X chromosome, which encodes the gap junction protein connexin 32 (Cx32). It has been postulated that, as CMT1X results from a loss of GJB1 gene function, affected males of a similar age will have a similarly severe neuropathy and hence similar CMTES scores. **Materials and Methods:** We retrospectively reviewed CMT Symptom Score (CMTSS), CMT Examination Score (CMTES), CMT Neuropathy Score (CMTNS), and neurophysiology in 20 male patients with CMT1X. **Results:** The CMTES varied prominently among males in each age range (21-30years: range 7-15, 31- 40yrs: 8-20, 41-50yrs: 10-20). The variation was predominantly due to differences in the sensory component of the neurological examination. Abductor Pollicis Brevis (APB) strength (median 2/5) was noticeably lower than the First Dorsal Interosseous (FDI) strength (median 4-/5). **Conclusions:** The wide variation in the CMTES scores suggests that, in addition to loss of *GJB1* function, yet to be identified epigenetic factors or environmental factors may contribute to the disability in CMT1X.

Poster 46
Referral patterns to a 4848glier4848z peripheral neuropathy clinic
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Objective: To determine the referral patterns and determine how many patients researched the clinic over the internet. **Background:** An expanding number of genetic causes for inherited neuropathy and therapeutic modalities for inflammatory neuropathies mean that patients are being increasingly referred to specialist neuropathy clinics, placing increasing demands on resources. Anecdotal experience suggests that patients may be partially driving this increased demand by researching clinics on the internet and requesting second opinions. This study aimed to audit referral patterns to a specialised peripheral nerve clinic and also whether patients had researched the clinic on the internet. **Materials and Methods:** New and follow-up patients at a specialised peripheral neuropathy clinic offering expertise in both genetic and acquired neuropathies were requested to fill a short questionnaire, indicating how they had been referred and if they had researched the clinic online. **Results:** The study is ongoing. 34% of the patients reviewed at the clinic were new patients. 93% were referred by other consultants and 7% by their general practitioner. 11% of the referrals were initiated by the patient requesting a second opinion. 24% of the patients indicated that they had researched the clinic on the internet. **Conclusions:** Results show most patients are referred by other consultants. It is not unusual for a patient to request another opinion having researched their illness online. A quarter of patients look up the clinic online before they attend, whether they had requested a second opinion or not.

Poster 47
Clinical and genetic characterisation of hereditary sensory neuropathy type 1 caused by mutations in SPTLC2

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Hereditary sensory neuropathy type 1 (HSN1) is an autosomal dominant predominantly sensory neuropathy with variable motor involvement and ulceromutilating complications. Mutations in *SPTLC1*, encoding the first subunit of the enzyme serine palmitoyltransferase, cause HSN1. More recently, mutations in SPTLC2, encoding the second subunit of serine palmitoyltransferase were described as causing HSN1 in four families. The pathomechanism is through a shift in subtrate specificity of the enzyme and accumulation of deoxysphingoid bases (DSBs) which are toxic to neurites in culture. We screened 107 patients with HSAN, negative for other genetic causes, for mutations in *SPTLC2*, and expressed the novel mutations found in human embryonic kidney (HEK) 293 cells. Two novel mutations were found in three unrelated families. The phenotype was similar to those previously described with HSN1, with adult-onset ulceromutilating sensory-predominant neuropathy; one family had disease onset in the first decade. All affected patients had elevated plasma levels of DSBs, the level of which correlated with neuropathy severity. HEK293 cells expressing both mutations were demonstrated to produce high levels of DSBs, supporting their pathogenicity. This study confirms the association of mutations in *SPTLC2* with HSN1 and suggests that the level of DSBs may correlate with the severity of the neuropathy, as has been suggested in patients with *SPTLC1* mutations.

Poster 48
A new phenotype of brain iron accumulation with dystonia, optic atrophy and peripheral neuropathy

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Background: Neurodegeneration with brain iron accumulation is clinically and genetically heterogeneous, being due to mutations in at least 7 nuclear genes. **Methods:** We performed homozygosity mapping and whole exome sequencing in two brothers with brain iron accumulation from a consanguineous family. **Results:** We identified a homozygous missense mutation in both brothers in the very recently identified chromosome 19 open reading frame 12 (*C19orf12*) gene. The disease presented before age 10 years with slowly progressive tremor, dystonia and spasticity. Additional features were optic atrophy, peripheral neuropathy and learning difficulties. A raised serum creatine kinase indicated neuromuscular involvement. A compensatory mitochondrial proliferation was suggested by respiratory chain enzyme activities and blue native polyacrylamide gel electrophoreses (PAGE) in myoblasts of a patient, implicating the possible role of mitochondrial dysfunction in the pathological mechanism. **Conclusions:** Further studies are needed to explore the function of the *C19orf12* gene, and extended genetic analysis on larger patient cohorts will provide more information about the presentation and frequency of this disease.

Poster 49
Genetic and Functional Investigation of Brown-Vialetto-Van Laere syndrome and Related Neuropathies

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Background: Brown-Vialetto-Van Laere (BVVL) syndrome and Fazio-Londe syndrome are part of a group of rare, generally recessive motor neuron diseases with early onset or onset in late childhood or early adulthood which present with cranial nerve palsies, sensorineural hearing loss, respiratory insufficiency and lower motor neuron signs. Mutations have been found in genes encoding riboflavin transporters, C20orf54 and GPR172B, leading to flavin deficiency. Riboflavin is necessary for the synthesis of FAD and FMN, which function as coenzymes in the mitochondrial respiratory chain (MRC) enzyme complexes I and II and therefore play a role in energy metabolism. FAD is also involved in mitochondrial fatty acid β oxidation and branched amino-acids catabolism.
Aims: The aim of this project is to understand how mutations in riboflavin transporters lead to neurodegeneration, as riboflavin deficiency has a very different clinical presentation.
Methods: We are sequencing the known genes in BVVL patients to identify new mutations as well as undertaking exome sequencing in patients without mutations in the known genes. To elucidate the pathomechanisms of neuronal death in BVVL, the riboflavin transporters are to be knocked down independently in SH-SY5Y cells. We will assess MRC function in the knockdown cells and patient fibroblasts by measuring the mitochondrial membrane potential and response to mitochondrial toxins.
Expected outcomes:
Exome sequencing was successfully used to identify the genetic cause of disease in a patient with BVVL-like motor neuron disease who had no detectable mutations in the known genes. Preliminary data suggests that the mitochondrial membrane potential may be maintained differently as a result of riboflavin transporter mutations. We will determine whether mitochondrial dysfunction may be one of the pathways leading to neuronal damage in BVVL.

Poster 50
Clinical, neuropathological and radiological evidence for a rare complication of rituximab therapy
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Introduction: We report a rare case of myofasciitis and meningitis with deafness caused by systemic echovirus infection in the setting of rituximab induced hypogammaglobulinaemia.
Case report: A 46 year old man was treated 4 years into Grade 1 follicular B lymphoma with R-CHOP chemotherapy achieving total remission on re-staging PET scanning. 1 year into maintenance therapy with rituximab, he developed myalgia, shoulder pain, fatigue, weight loss and progressive hearing and balance impairment. Examination revealed subtle hearing loss, upper limb fasciculations and reduced limb reflexes. Investigations showed panhypogammaglobulinaemia, normal creatine kinase, raised CSF protein and white cells with absence of clonality on typing by flow cytometry and PCR studies. A recurrence of lymphoma was excluded. Neurophysiology showed a predominantly sensory neuropathy and mild myopathic changes. Audiometry confirmed sensorineural deafness and vestibulocochlear failure. An axial STIR MRI of the thighs showed florid myofascial high signal consistent with fasciitis. A muscle biopsy of the left vastus lateralis confirmed florid myofasciitis with sheets of CD68+ macrophages and perivascular inflammation in the fascia and perimysium, and absent B cells. Enterovirus (Echovirus type 9) was identified by PCR in the serum and CSF, but not in muscle. Treatment with IVIG lead to sustained rise in IgG levels, and serum and CSF were

negative for echovirus at 6 and 10 weeks. The muscle pain and fasciculations resolved and repeat imaging was normal at 16 weeks. A sudden deterioration lead to total deafness at 20 weeks. CSF showed viral reactivation with increased cells and protein. The dose of IVIG was increased and led to viral clearance and reduced inflammation in the CSF.
Conclusions: Our case highlights the long term immunomodulatory effects of rituximab therapy causing increased susceptibility to rare viral infections. The rare complication of enterovirus myofasciitis is reported and its pathology is documented clearly for the first time.

Poster 51

Genetic dysfunction of *MT-ATP6* can cause axonal Charcot-Marie-Tooth disease

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Objective: Charcot-Marie-Tooth (CMT) disease is the commonest inherited neuromuscular disorder affecting 1 in 2,500 individuals. However, primary mitochondrial DNA (mtDNA) mutations are not generally considered within the differential diagnosis of patients with uncomplicated inherited neuropathy, despite the essential requirement of mitochondrial respiratory chain ATP for normal axonal function. We identified the pathogenic mtDNA mutation m.9185T>C in *MT-ATP6* at homoplasmic levels in a family with mitochondrial disease in whom a severe motor axonal neuropathy was a striking feature. This led us to hypothesise that this mutation might be an unrecognised cause of isolated axonal CMT and distal hereditary motor neuropathy (dHMN).
Methods: 447 unrelated probands with CMT2 (275) and dHMN (172) were screened for m.9185T>C following exclusion of mutations in most known CMT2/dHMN genes. Mutation load in positive cases was quantified using restriction endonuclease analysis and Blue-native gel electrophoresis (BN-PAGE) to analyse the effects of m.9185T>C on complex V structure and function.
Results: Three further families, all with CMT2, were found to 51uglie the m.9185T>C mutation. Patients could be broadly classified into four groups of increasing clinical severity according to their mutant m.9185T>C levels. Blue-native gel electrophoresis demonstrated both impaired assembly and reduced activity of the complex V holoenzyme as a direct consequence of the m.9185T>C mutation.
Interpretation: We have shown that m.9185T>C in *MT-ATP6* causes CMT2 in 1.1% of genetically undefined cases. This finding has major implications for diagnosis and genetic 5151glier5151 of patients with CMT2. Recognition that mutations in *MT-ATP6* cause CMT2 enhances current understanding of the pathogenic basis of axonal neuropathy and should translate into new research and therapeutic strategies for patients with inherited neuropathy.

Poster 52

Strengthening Hip Flexors to Improve Walking Distance in People with Charcot-Marie-Tooth Disease

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Previous walking studies of people with Charcot-Marie-Tooth (CMT) disease revealed that they utilise a proximal adaptive gait strategy to compensate for distal impairments. We demonstrated that fatigue of the hip flexor muscles can limit walking distance in prior studies. The aim of this single blinded cross over study was to investigate the effect of a 16 week training programme to increase hip flexor muscle strength. This was measured by fixed myometry at four angles: 0º, 20º, 45º and 90º. Additional measures of walking endurance, gait, speed, exertion, fatigue and general activity were also recorded. Thirty two people with different types of CMT were recruited with a mean age 43.5 (±14.76), 17 male, 15 female and a median CMTES score of 10. Twenty eight people finished the study, completing 93% of the prescribed exercises. Hip flexor strength showed a significant effect of training of the left hip flexor (p= 0.041) but not the right (p=0.19). No significant improvements were observed in the secondary measures. A significant negative correlation was noted between the baseline hip flexor strength at zero degrees and the change with training (r=-0.44, p=0.018) indicating greater strengthening effect in people who were weaker at baseline. No negative effects were observed. The variability of response could have been due to genetic heterogeneity of the sample or variation of participation in exercise prior to the study. Low numbers may have lead to the sample's poor functional carry over, or perhaps a lack of functional training as part of the intervention.

Poster 53

A novel p.glu175x premature stop mutation in the C-terminal end of HSP27 is a cause of CMT2

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Mutations in the gene HSPB1, encoding the small heat shock protein 27 (HSP27), are a cause of distal hereditary motor neuropathy (dHMN) and autosomal dominant axonal Charcot-Marie-Tooth disease (CMT2). DHMN and CMT2 are differentiated by the presence of a sensory neuropathy, although this division is misleading as patients with dHMN (like those with amyotrophic lateral sclerosis (ALS)) eventually develop sensory involvement. It has recently been published that mutations in the C-terminus of HSP27 cause dHMN and those at the N-terminus CMT2. We present a family with a novel mutation in the C-terminus of HSP27 with a motor predominant distal neuropathy and sensory involvement that we classified as CMT2. This case highlights the artificial distinction between patients with motor predominant forms of CMT2 and dHMN and argues against the hypothesis that mutations in the C-terminus have no sensory involvement.

Mitochondrial Disease

Poster 54

Novel *SDHA* and *SDHB* mutations as a cause of isolated mitochondrial complex II deficiency

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Isolated complex II deficiency represents one of the rarest forms of mitochondrial disease, accounting for just 2% of all respiratory chain deficiency diagnoses. Entirely nuclear-encoded, the succinate dehydrogenase (SDH) genes (*SDHA*, *SDHB*, *SDHC* and *SDHD*) encode the four structural subunits of complex II, whilst there are two known assembly factors (*SDHAF1* and *SDHAF2*). Only a handful of reports describe inherited *SDH* gene defects as a cause of mitochondrial disease, involving *SDHA* and *SDHAF1* in paediatric presentations dominated by either cardiomyopathy (*SDHA*) or Leigh syndrome (*SDHAF1*). Interestingly, all four SDH genes and *SDHAF2* have tumour suppressor function, with numerous germline and somatic SDH mutations associated with an increased risk of hereditary phaeochromocytoma and paraganglioma syndromes.

Here, we report the clinical and molecular investigations of two patients referred to our service with suspected mitochondrial disease. Histochemical and biochemical analyses revealed an isolated complex II deficiency in both cases which we show are due to novel mutations within structural *SDH* genes; the first patient presented with cardiomyopathy and leukodystrophy due to compound heterozygous *SDHA* mutations, whilst a second patient presented with hypotonia and leukodystrophy due to a novel, homozygous *SDHB* mutation. Western blotting and BN-PAGE studies revealed decreased levels of the relevant SDH subunit expression and complex II assembly, establishing the pathogenicity of the *SDHA* and *SDHB* mutations. This report represents the first example of *SDHB* mutation as a cause of mitochondrial respiratory chain disease.

Poster 55

***MPV17* mutation causes neuropathy and leukoencephalopathy with multiple mtDNA deletions in muscle**

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Disorders of mitochondrial DNA (mtDNA) maintenance are clinically and genetically heterogeneous, embracing recessive mtDNA depletion syndromes affecting children and adult-onset multiple mtDNA deletion disorders.

Here we show that mutation of *MPV17* – a gene implicated in severe, infantile hepatocerebral mtDNA depletion disorders characterised by a loss of mtDNA copies – can also cause clonally-expanded mtDNA deletion and focal cytochrome *c* oxidase (COX) deficiency in skeletal muscle associated with an adult presentation of neuropathy and leukoencephalopathy. These data confirm that the mpv17 protein is intimately involved in both the mtDNA replication and repair processes and that autosomal recessive mutations in this gene are associated with both quantitative and qualitative mtDNA abnormalities.

Poster 56

Studying the molecular basis of the reversibility in infantile reversible cytochrome *c* oxidase deficiency

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Infantile reversible cytochrome *c* oxidase (COX) deficiency is an exceptional mitochondrial disorder by showing spontaneous recovery if infants surviving the first months of life. Our group has identified the homoplasmic m.14674T>C mt-tRNA^{Glu} mutation as a cause of this disease. Although previous results provide evidence for a pathogenic role of m.14674T>C, do not explain why all patients develop severe isolated myopathy in the neonatal period and what triggers the timed recovery.

Recently, another reversible disease has been discovered with infants (infantile reversible hepatopathy) caused by mutations in the TRMU gene. Based on the similar age-dependence and reversibility we investigated whether a reduced thiolation may aggravate the clinical manifestation in reversible COX deficiency. When the thiolation pattern of different tRNA species was compared in cells of reversible COX and TRMU patients, we found that the thiolation of mt-tRNA^{Glu} and mt-tRNA^{Lys} was reduced in both cell lines. Moreover, downregulation of TRMU in the patient's cells showed an even more decreased steady-state of mt-tRNA^{Glu}; indicating that am impaired thiolation may contribute to the disease.

We also investigated the developmental expression of tissue specific COX VI/VII isoforms in mice to define whether changes in the expression of these isoforms early in life may contribute to the spontaneous recovery in reversible COX deficiency. The liver type isoforms gradually decreased, and the heart/muscle-type isoforms increased through development in the first weeks of life, confirming an age-dependent isoform switch.

The better understanding of compensatory factors contributing to spontaneous recovery in mitochondrial disease may offer clues towards molecular therapies.

Poster 57
Mitochondrial DNA deletions do not have a replicative advantage in human muscle
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The accumulation of mitochondrial deletions (mtDNA) by a process known as 'clonal expansion' is important in determining clinical severity in many cases of inherited and sporadic mitochondrial disease, as well as contributing to focal respiratory chain deficiency in both ageing and neurodegenerative disorders. The mechanism involved in clonal expansion of mtDNA deletions remains unknown, although 3 main hypotheses have been proposed. Of these theoretical mechanisms of clonal expansion, one proposes that random drift is sufficient to cause mtDNA deletion accumulation, while the remaining two both propose that an element of selection is involved. The 'advantage of the slowest' mechanism theorises that the inherent dysfunction of deleted mtDNA species causes them to be positively selected for due to a low ROS production, but for this study we wished to test the 'advantage of the smallest' hypothesis. This mechanism proposes that smaller mitochondrial genomes possess a replicative advantage; we chose to investigate this by determining whether smaller (deleted) mitochondrial genomes spread further by clonal expansion through muscle fibres than larger mtDNA molecules. We characterised mtDNA deletion sizes in 60 'long' and 60 'short' COX-deficient skeletal muscle fibres from patients with mtDNA maintenance disorders, using long extension PCR and breakpoint sequencing. No significant difference was found in mtDNA deletion size between these groups, suggesting that smaller mitochondrial genome sizes do not confer a replicative advantage and therefore this is not responsible for clonal expansion of mtDNA deletions – potentially ruling out the 'advantage of the smallest' hypothesis as a mechanism to drive clonal expansion.

Poster 58
Development of the Pronuclear Transfer Technique to Prevent Transmission of Mitochondrial DNA Disease in Humans
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Mitochondrial DNA (mtDNA) diseases are an important cause of muscle disease and are often caused by mutations in mitochondrial genes. MtDNA is strictly maternally inherited and so a woman with mtDNA disease due to a mitochondrial DNA mutation is at significant risk of passing the defect to her children. We have demonstrated that the technique of pronuclear transfer between human embryos has the potential to prevent this transmission. The results are very promising but further studies must be performed to ensure the safety and efficacy of the technique before it can be considered for clinical use.
One issue we are currently investigating is the presence of the polar bodies during the pronuclear transfer procedure. These small cells contain DNA from the oocyte and are found adjacent to the embryo membrane following fertilisation. Our concern is that introduction of the pronuclear karyoplast with the fusion agent during pronuclear transfer may also result in fusion of the polar bodies with the recipient embryo. To overcome this, we are optimising a technique to remove the polar bodies from the embryo prior to pronuclear transfer. Our initial results suggest that this should be possible as pronuclear transfer embryos which have undergone polar body removal are capable of onward development in culture.

Poster 59
Evidence of early cardiac impairment in m.3243A>G mutation carriers

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Background Cardiomyopathy is an independent predictor of morbidity and early mortality in mitochondrial disease, and occurs in 20-40% adult patients harbouring the common m.3243A>G mutation, usually with a hypertrophic phenotype. To date, no detailed study of myocardial structure and function has been performed in m.3243A>G mutation carriers to identify antecedent markers of cardiac involvement.
Methods Cardiac magnetic resonance imaging was performed in 20 adult patients (10 males, mean age 38.7 ± 13.1 years) harbouring the m.3243A>G mutation, without clinical evidence of cardiac involvement, and 20 age- and gender-matched healthy controls (10 males, 38.4 ± 14.2 years). Mutation load was determined in urine and disease burden was assessed using the Newcastle Mitochondrial Disease Adult Scale (NMDAS).
Results Compared to controls, patients had increased left ventricular mass index (LVMI) and LV mass to end-diastolic volume ratio (M/V ratio), peak torsion, and torsion to endocardial strain ratio (TSR); longitudinal shortening was decreased in patients, and this occurred in association with increased LVMI (r=-0.55, p=0.01). Among patients there was no significant correlation of LVMI or M/V ratio with age, blood pressure or fasting blood glucose; urinary mutation load and NMDAS score were strongly associated with LVMI (r=0.75, p<0.001 and r=0.82, p<0.0001 respectively).
Conclusions Early concentric cardiac remodeling is identifiable in patients harbouring the m.3243A>G mutation without clinical cardiovascular disease. Patients with higher mutation loads and disease burden may be at increased risk of developing cardiomyopathy. The identification of early cardiac impairment may enable the initiation of timely medical intervention and prevention of progressive cardiomyopathy.

Poster 60

Processing Speed is impaired in patients with Mitochondrial Disease

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Introduction*:** Mitochondrial diseases are a diverse group of genetic disorders characterised by genotypic and phenotypic heterogeneity. Whilst there is significant clinical variability, neurological impairment remains one of the hallmarks of mitochondrial disease and cognitive impairment is one of the least understood aspects of this. Moreover, processing speed which is an important, low level cognitive ability has recently been noted to be impaired in mitochondrial diseases. ***Aims*:** To systematically investigate processing speed in a large cohort of patients with mitochondrial disease. ***Methods: Cognitive data were recorded using the WTAR, Symbol Search from the WAIS-III and the Speed of Comprehension test from the SCOLP in patients attending the NHS Specialised Services Clinic in Newcastle. One hundred and ninety-one patients from four genotypes (m.3243A>G mtDNA point mutation; m.8344A>G mtDNA point mutation; single mtDNA large scale deletion and multiple mtDNA deletions) were assessed. *Results*: Adjusting for peak ability using the WTAR, processing speed is impaired and declined with age, and disease severity. Genotype had no impact on processing speed ability. ***Conclusion**:* Slowed processing speed is an important contributor to cognitive and functional difficulties, in patients with mitochondrial diseases. As such, it is essential to understand the cognitive difficulties facing patients with mitochondrial disease in order to allow

careful planning of future care, patient education and caregiver support.

Poster 61

Expanding the phenotypic and genotypic spectrum of adult *RRM2B*-related mitochondrial disease

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Introduction: Defects in *RRM2B* are now recognized as an important cause of nucleotide metabolism dysfunction that causes mtDNA instability resulting in mitochondrial disease. Despite the emergence of *RRM2B*-related disease as the third most common cause of multiple mtDNA deletions, following *POLG* and *C10orf2* (Twinkle) mutations, the clinico-pathological features of adults with *RRM2B*-related mitochondrial disease has yet to be fully established.
Methods/Aims: We reviewed the clinical and histopathological findings of 27 unrelated adult patients (23 families) with genetically-confirmed autosomal dominant and autosomal recessive *RRM2B* mutations (including novel mutations) to i) understand the clinical spectrum of adults with *RRM2B*-related mitochondrial disease, and ii) establish the relationship between the clinical phenotype and underlying genetic defect.
Results: Progressive external ophthalmoplegia (PEO) was universal and often associated with ptosis. Proximal muscle weakness, bulbar dysfunction and fatigue were the other predominant clinical features. Hearing loss and gastrointestinal disturbance were frequently seen. There was a clear relationship between phenotype and genotype.
Conclusion: We demonstrate a clear clinical correlation between autosomal recessive *RRM2B* disease which causes a severe childhood onset multisystem disorder, and autosomal dominant *RRM2B* disease that is associated with a milder adult onset myopathic phenotype. The prominence of bulbar dysfunction, hearing loss and gastrointestinal problems, would support early prioritization of screening the *RRM2B* gene over *POLG* and *C10orf2* in adult patients with PEO and multiple mtDNA deletions.

Poster 62

Resistance training in patients with mitochondrial myopathy

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Exercise training has been found to be beneficial in patients with mitochondrial myopathy which is caused by mutations within the mitochondrial genome leading to defects in the mitochondrial electron transport chain. These defects result in muscle weakness and exercise intolerance and even though the benefits have been documented, it still remains unclear whether patients with mitochondrial myopathies should be encouraged to exercise and how much exercise is beneficial. The rationale for strength training within our patients is to induce satellite cell activation and encourage regeneration of muscle by satellite cells which have low or absent levels of mutated mtDNA therefore shifting the genetic balance within the muscle fibres resulting in a beneficial change in the biochemical phenotype.
The aims of this study were to determine the ability of resistance muscle strength training to improve skeletal muscle strength and confirm that resistance training in patients with mitochondrial abnormalities improves exercise tolerance, oxidative capacity and quality of life;
Eight patients (six men; mean age 41 years (29-57 years)) with single large scale mtDNA deletions underwent resistance exercise training 3 times a week for 16 weeks. Training consisted of leg extensions, leg presses, calf raises, and hamstring curls. Muscle biopsies, Physiological tests, and

quality of life tests were performed pre and post resistance training. The muscle biopsies were taken to assess biochemical and genetic changes, in order to do this we utilised a variety of techniques which included real time PCR and histochemical staining procedures.
After 16 weeks of resistance training we found an improvement in oxidative capacity, improved muscle strength, improved quality of life and changes within the patients' muscle biopsies. These data show that resistance training is a potential treatment for patients with mitochondrial myopathies and is well tolerated within our patient group.

Poster 63
Long term endurance training and deconditioning in patients with mitochondrial myopathy
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Mitochondrial myopathies are an important group of progressive muscle disorders caused by mutations in the mitochondrial genome (mtDNA). Mutations within mtDNA lead to defects in the mitochondrial electron transport chain which in turn can result in exercise intolerance, fatigue, muscle weakness, severe disability or even death. There has been significant progress in the management and diagnosis of patients with mitochondrial myopathy, however there still remains a need for effective treatment.
The aim of this study was to assess if long term endurance training, by promoting mitochondrial biogenesis and expansion of wild type mitochondrial DNA copy number, would benefit patients with mitochondrial myopathy therefore providing a safe and effective therapy for our patients. We also aimed to investigate if deconditioning, resulted in a lowering of wild type mtDNA copy number therefore having a detrimental effect.
Patients with point mutations or single deletions within their mtDNA are undergoing endurance training and being assessed at baseline, 26 weeks, 1 year and 2 years. Patients underwent the deconditioning stage between either baseline and 26 weeks or 26 weeks and 1 year. The primary outcome measures include physiological assessment of muscle oxidative capacity as well as genetic, biochemical and molecular measures.
Here we present data relating to the effect of long term endurance exercise training and combined deconditioning in patients with mitochondrial myopathy highlighting the biopsy changes in mitochondrial deletion levels as determined by real time PCR and pyrosequencing; mitochondrial wild-type and total copy number as determined by real time PCR; changes in the biochemical phenotype of the muscle using histochemical staining techniques. We discuss changes in the numbers of intermediate muscle fibres and show the application of a novel technique for characterising the intermediate fibre.

Poster 64
The Medical Research Council Neuromuscular Centre for Translational Research Mitochondrial Disease Patient Cohort Study UK: from conceptualisation to utilisation
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Background: Mitochondrial disease is a clinically heterogeneous debilitating condition with a minimum birth prevalence of 9.2 per 100,000, and is associated with a reduced life expectancy. The variable clinical phenotype has previously hindered systematic studies and natural history studies

are largely anecdotal. There is currently no evidence-based treatment for mitochondrial disease. **Objectives:** To develop a cohort of 1000 people from across the UK with biochemically, and/or genetically, confirmed mitochondrial disease to facilitate large-scale interventional trials of drugs and novel treatments, and to assess prevention strategies. The cohort will also allow definitive studies on the transmission of mtDNA mutations, and provide longitudinal data on the natural history of the disease. **Design & Methods:** Following consultation with the clinical research leads, the Bioinformatics Department at Newcastle University designed and accommodated a secure database on a stand-alone dedicated Dell PowerEdge R300 server. The database is backed-up daily to a second identical, but independent server. A data custodian was appointed to oversee the maintenance, security and access to stored patient information. Research associates were employed in Newcastle and London, along with administrative support, to identify and recruit patients to the cohort study. NIHR portfolio adoption facilitated recruitment through local principal investigators at an additional 16 sites across the UK. The British Neurology Surveillance Unit and British Paediatric Neurology Surveillance Unit have been utilised to identify patients not under active follow-up at one of the three centres (Newcastle, London and Oxford) providing the NHS Highly Specialised Service for Rare Mitochondrial Diseases in Adults and Children. Written informed consent is obtained from patients with confirmed biochemical, and/or genetic, mitochondrial disease. Clinical data is recorded retrospectively from medical notes, and prospectively when the patient is reviewed in a clinical setting. The Newcastle Mitochondrial Disease Assessment Rating Scale is utilised whenever possible. All patient data is entered anonymously and unique identifiers are used to contact patients suitable for clinical studies. The Mitochondrial Disease Oversight Committee (MDOC), which includes an ethicist and a patient representative, was established to evaluate the scientific merit and appropriateness of applications and govern utilisation of the Cohort. **Results:** 870 people have been consented to the cohort to date with recruitment continuing in line with proposed targets; clinical data is available on 729 (84%). 50% harbour mtDNA pathogenic mutations, 8% nDNA mutations, and 42% have confirmed biochemical defects but no genetic aetiology identified to date. The m.3243A>G mtDNA mutation is the most commonly identified genetic mutation in this cohort. At present 7% are clinically asymptomatic, 57% exhibit neurological features, 29% have ophthalmological involvement, 19% have cardiac involvement, 19% have gastrointestinal disturbances, 12% suffer from endocrine dysfunction. 12 clinical studies have been approved by MDOC, involving over 250 patients with mitochondrial disease. 5 clinical guidelines have been produced in the last year, which are available for download from the internet: www.mitochondrialncg.nhs.uk. **Conclusion:** For the first time in the UK it is now possible to access a large cohort of well-characterised patients with mitochondrial disease. The cohort provides objective data on mitochondrial disease progression, in children and adults, allowing evidence-based guidelines to be developed, and prognostic advice to be provided to patients and families. There is a vast amount of data still to be analysed that will provide systematic evidence and allow the development of disease prevention strategies.

Poster 65

Improving clinical trials evaluation: physiological and functional correlates in mitochondrial disease

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Introduction: Mitochondrial diseases are one of the most common forms of inherited neuromuscular disease. The presentations of these diseases are highly variable with neurological and systemic involvement. An improved understanding of the relationship between physiological and functional measures with disease severity will assist in the evaluation of performance in clinical trials.
Methods: 24 people with genetically identified mitochondrial disease underwent: assessment of disease severity using the Newcastle Mitochondrial Disease Adult scale (NMDAS), mitochondrial mutation load (%). Maximal aerobic capacity (VO_{2MAX}) was determined using a maximal progressive exercise test. Functional ability was assessed using a timed up and go test (TUG) previously used to test basic mobility skills in the elderly population.
Results: All participants completed all of the tests (age 18-59, mutation load 22-95, mean NMDAS-23). Clinical severity, assessed using the NMDAS, was positively associated with maximal aerobic

capacity (VO_{2MAX} r=0.5, p=0.04) and functional ability (TUG; r=0.5,p=0.02). Maximal aerobic capacity was, in turn, associated with functional ability (r=0.4,p=0 04).
Conclusions: Physiological and functional outcome measures are potential surrogate markers for disease severity in mitochondrial disease and may assist in providing an objective means of assessment performance. The use of these measures may be useful in measuring effect on disease in future intervention studies and should be further explored.

Poster 66
Systematic review of controlled trials in the treatment of mitochondrial disorders
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Background: Mitochondrial respiratory chain disorders are the most prevalent group of inherited neurometabolic diseases. Current treatment is supportive, although some treatments have reported disease-modifying efficacy in isolated cases and small clinical trials. This review was carried out to evaluate the efficacy of these treatments.
Methods: We searched various databases for clinical trials in mitochondrial disease up to the end of 2009. We included only controlled trials (including crossover studies). Studies with a high risk of bias were excluded. Interventions included any pharmacological agent, dietary modification, nutritional supplement, exercise therapy or other treatment. Details of the number of randomised patients, treatment, study design, study category, allocation concealment and patient characteristics were extracted. Analysis was based on intention-to-treat data.
Results: 1335 abstracts were reviewed, and from this 19 abstracts were identified for further review. Upon detailed review, ten studies fulfilled the entry criteria. Three trials used creatine monohydrate alone, with one reporting improved measures of muscle strength and post-exercise lactate. One trial studied the effects of a combination of co-enzyme Q10, creatine monohydrate, and lipoic acid, and reported an improvement in surrogate markers of disease activity. Five trials studied the effects of dichloroacetate: three trials in children showed an improvement in secondary outcome measures of mitochondrial metabolism, one trial of short-term therapy in adults demonstrated no clinically relevant improvement, and one longer-term trial in adults was terminated prematurely due to adverse effects. One trial using dimethylglycine showed no significant effect.
Conclusions: There is currently no clear evidence supporting the use of any intervention in mitochondrial disorders. Further research is needed to establish the role of a wide range of therapeutic approaches.

Poster 67
Changes in mitochondrial function over time and with exercise in patients with mitochondrial disease
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Mitochondria are vital to the production of ATP within cells and the machinery responsible for this- the respiratory chain- is partly encoded for by mitochondrial (mt) DNA which are present in multiple copies. Patients with mitochondrial disease due to mtDNA defects deteriorate clinically, albeit, at varying rates. In order to improve our understanding of mtDNA disease progression, we have studied a cohort of adult patients all of whom harbour a known defect of mtDNA: single large-scale mtDNA deletion. The defect causes chronic progressive external ophthalmoplegia, ptosis, muscle weakness and fatigue commonly in this cohort of patients.
The gold standard diagnostic technique for the disease is muscle biopsy where respiratory chain deficiency is indicated by the presence of cytochrome *c* oxidase (COX)-deficient cells. These have also been found at a lower level in healthy aged muscle. We have 59uglier on muscle respiratory chain deficiency as a marker of muscle weakness progression by using a novel densitometric approach. Exercise therapy has been proposed to improve muscle biochemistry by increasing mtDNA copy number. We aimed to investigate this by studying multiple quadriceps muscle biopsies taken from patients within our cohort who have been involved in exercise trials.

Muscle biopsy biochemical findings showed a worsening of the biochemical defect over time in some patients and also a response to both exercise and detraining. Molecular genetic analyses documented changes in mtDNA copy number over time but no significant change in the deleted mtDNA level. Further studies are required to confirm these observations given the implications on recommended therapeutic interventions.

Poster 68

***MFN2* mutations cause compensatory mitochondrial DNA proliferation**

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Mitofusin 2 (MFN2) is an essential protein for mitochondrial outer membrane fusion and maintenance of mitochondrial network. In this study, we investigated the effect of pathogenic *MFN2* mutations on mitochondrial DNA (mtDNA) copy number in peripheral blood leukocytes of 58 individuals. We observed significantly greater mtDNA proliferation in patients harbouring *MFN2* mutations compared with age-matched controls. This observation is likely to reflect a compensatory proliferative response to an underlying fusion or OXPHOS defect. We also determined the histochemical and molecular features present in a skeletal muscle biopsy from a 61-year-old *MFN2*-positive patient. Dual cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) histochemistry revealed a low level of COX-negative muscle fibres (<0.7%). Myofibrillar ATPase histochemical staining demonstrated increased muscle fibre atrophy and pathological fibre-type grouping. Single muscle fibre analysis revealed high levels of mtDNA deletions in COX-negative fibres and significant mtDNA proliferation in both COX-negative and COX-positive fibres when compared with normal control muscle fibres. This finding is consistent with the mtDNA proliferation observed in peripheral blood leukocytes.

Poster 69

A proposed consensus panel of organisms for determining evolutionary conservation of mt-tRNA point mutations

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Point mutations in the 22 mitochondrial (mt-) tRNAs are responsible for a wide spectrum of disease phenotypes including myopathy, epilepsy, diabetes and deafness. Mitochondrial disease can occur in both children and adults, with high-energy demanding tissues such as the brain and skeletal muscle (SKM) most commonly affected. Determining pathogenic mt-tRNA variants from harmless polymorphisms is crucial for providing accurate genetic 6060glier6060 to families in the clinic. However, assigning pathogenicity to mt-tRNA point mutations is complex, and requires a structured evaluation of multiple strands of evidence. This evidence includes determining mutation heteroplasmy, histochemical and biochemical studies as well as functional studies that investigate changes in mt-tRNA steady-state levels and mutation segregation with biochemical deficiency. Evolutionary conservation is often considered mandatory, but lack of a standard panel of organisms to assess conservation complicates comparison between reports and undermines the value of conservation-based evidence. This is illustrated by the alarming degree of variation in both the number of different species selected for determining conservation across all available reports of mt-tRNA variants and the taxonomic range of species selected by any individual report. We have demonstrated that intra-species mt-tRNA sequence variation is sufficiently low for sequence data from a single organism to adequately represent a species and propose a standardised panel of organisms for conservation assessment of mt-tRNA variants. We describe the integration of this conservation panel into the pathogenicity scoring system designed to assess mt-tRNA variation associated with mitochondrial disease.

Congenital Myopathies, Limb Girdle Myopathies & IBM

Poster 70

Investigating the effects of pharmacological up-regulation of the heat shock response 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 disease has long been considered an immune-mediated disorder, recent studies indicate a partly myodegenerative process in sIBM muscle. In particular, there is evidence for abnormal protein aggregation in sIBM muscle, with aggregates incorporating well-recognised proteins including amyloid-beta precursor protein (β-APP), amyloid-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 misfolded proteins. HSP up-regulation has been demonstrated to be a potent therapeutic strategy in cellular and animal models of neurodegenerative disease.
In this study, we examined the effects of up-regulating the HSR in an *in vitro* model of sIBM. Using primary muscle cultures derived from neonatal rats we found that over-expressing β-APP by gene transfection results in the formation of cytoplasmic inclusion bodies that are immuno-reactive for ubiquitin, phosphorylated tau, p62, Hsp70 and TDP-43. B-APP transfected cells were found to have increased cytotoxicity and led to the translocation of TDP-43 from the nucleus to the cytoplasm. Proteasomal and mitochondrial abnormalities were also detected in β-APP over-expressing cells. However, treatment with Arimoclomol, a pharmacological co-inducer of the HSR not only reduced inclusion body formation but also increased cell survival and attenuated TDP-43 translocation and mitochondrial abnormalities. These results therefore suggest that targeting of the HSR may be of therapeutic benefit in sIBM.

Poster 71

A single in-frame deletion in the CAPN3 gene is linked to muscular dystrophy with a dominant pattern of inheritance

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The CAPN3 gene on chromosome 15q15 encodes calpain 3, a skeletal muscle-specific protease. Mutations in CAPN3 gene cause Limb-Girdle Muscular Dystrophy (LGMD) type 2A. Albeit a rare disease, LGMD2A is overall the most common form of autosomal recessive LGMDs. Several sporadic patients have been reported carrying a single mutation in CAPN3. In the absence of family history of neuromuscular disease, these findings are usually attributed to incomplete mutation screening. A recent report has identified dominant inheritance in three northern European families in whom only one mutation, a 21 bp in-frame deletion (c.643_663del21; p.Ser215_Gly221del), was identified. We report three further unrelated UK families harbouring the same heterozygous mutation. C.643_663del21 segregated with the disease in multiple generations with a dominant inheritance pattern. Similarly to that already reported, affected individuals showed an onset in the late teens-early 20s, raised CK (>1000) and mild muscle weakness with slow progression of disease. Muscle biopsies presented myopathic features and calpain 3 was greatly reduced on Western blot. In conclusion, we provide further evidence of a pathogenic role of c.643_663del21 in a mild form of muscular dystrophy with reduction of calpain 3.

Poster 72

Characterisation of novel ANO5 antibodies

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The ANO5 gene is mutated in LGMD2L and a non-dysferlin Miyoshi myopathy, MMD3. Recessive ANO5 mutations are associated with sarcolemmal lesions and defective membrane repair. In European patients the ANO5 mutation, c.191dupA, is present in both MMD3 and LGMD2L patients and is emerging to be a common cause of adult onset muscular dystrophy.
ANO5 belongs to the anoctamin protein family, 10 human proteins (ANO 1-10) sharing a similar structure consisting of eight transmembrane domains, a re-entry loop domain and the novel DUF590 domain. Recent studies have shown that ANO1 and ANO2 function as calcium activated chloride channels (CaCCs), which are ion channels gated by increases in intracellular Ca^{2+} concentration. Heterologous expression of ANO5 and other anoctamins has shown that not all anoctamins are CaCC's. For instance, ANO6 regulates the asymmetry of plasma membrane phospholipids. The function of ANO5 is not known as yet. Its biochemical characterization in muscle has been limited due to lack of specific and freely available antibodies. We have generated monoclonal antibodies to ANO5 using specific N-terminal and C-terminal peptides. We are currently characterising the hybridomas which detect overexpressed ANO5 fusion proteins in C2C12 cells. Once the validation tests complete successfully, the novel antibodies for ANO5 will provide an important resource for diagnostics and research.

Poster 73

A histological evaluation of protein accumulation in inflammatory myopathies

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Background The current diagnostic criteria for inclusion body myositis (IBM), first published in 1995, comprise pathological and clinical elements. The pathological features were considered to be specific to IBM but some features are also reported in other muscle disorders. Furthermore, in many clinically typical cases some pathological features may be absent. Several proteins associated with neurodegenerative diseases have been reported to accumulate in muscle fibres in IBM. The benefit of staining for these proteins to differentiate IBM from other myopathies is uncertain.
Aims To determine the sensitivity and specificity of immunohistochemical staining (IHC) for proteins associated with neurodegenerative diseases in IBM.
Methods Cases were selected from the archives of the John Radcliffe Hospital, Oxford or the National Hospital for Neurology and Neurosurgery, London. The case cohort included; IBM (n=17), steroid responsive myositis (n=5) and protein accumulation myopathies (n=11).
Frozen sections of muscle were stained using Congo red and IHC employing the following antibodies: p62, TAR DNA-binding protein-43 (TDP-43), amyloid β, α-synuclein, tau or fused in sarcoma protein (FUS), ubiquitin and myotilin. The number of fibres containing inclusions was quantitated using image analysis.
Results In IBM protein inclusions were most commonly labelled with antibodies to p62 and TDP-43. IHC demonstrated protein accumulation using antibodies to myofibrillar proteins, desmin and myotilin, and alpha B-crystallin. However, staining for β-amyloid, α-synuclein, tau or FUS was absent.
Conclusion Proteinaceous inclusions in IBM were most readily identified using p62 IHC suggesting that in routine practice this is a reliable marker for use in the diagnosis of IBM.

Poster 74

The natural history of sporadic Inclusion Body Myositis: data from the IBM-Net prospective cohort study

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Objective: To assess the clinical features and clinical course of sporadic inclusion body myositis (sIBM) in a prospective cohort of patients diagnosed with clinically or pathologically defined sIBM, according to Hilton-Jones criteria.
Methods: Clinical data, manual muscle testing, myometry (Cybex dynamometer) and IBM functional rating scale (FRS) were collected according to a 6363glier6363ze protocol (IBM-Net) at baseline (n=51) and 1-year follow-up (n=19).
Results: The male:female ratio was 2:1, mean age at examination was 68 years (range 44-95) and at disease onset 58 (37-91). The mean latency between symptom onset and IBM diagnosis was 4 years. Only 39% of patients were initially referred for suspected sIBM, the other 61% had various initial suspected diagnoses including polymyositis, statin myopathy, ALS and peripheral neuropathy. Proximal asymmetrical weakness of lower limbs and grip weakness were the most common reported symptoms at onset. Falls represented the initial complaint in 24% of patients. Mean FRS at baseline was 27/40 (range 15-38). We found a high frequency of dysphagia, with 50% of subjects complaining of swallowing difficulties. The clinical evaluation and myometry consistently showed that finger flexors, knee extensors and foot dorsiflexors were the most severely affected muscles. A subgroup of 19 patients had a second evaluation after one year, showing a mean loss of muscle strength at manual muscle testing of 2.9% ($p=0.02$) at manual muscle testing and 2.0% ($p=0.03$) at quantitative muscle testing and a mean reduction in IBM-FRS of 11% ($p<0.001$).
Conclusions: This study describes one of the largest cohorts of IBM patients reported so far, providing crucial information to correctly define the features and natural history of sIBM and to adequately design future clinical trials.

Poster 75

Observations on oligo-based therapy for Myotonic Dystrophy

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Recent work[1, 2] points to the potential of oligo-based therapies for the treatment of Myotonic Dystrophy. There are many different kinds of chemically modified oligonucleotides (CMOs) that could in principle be applied as a treatment for DM1 and DM2. In order to assess the therapeutic usefulness of different types of CMO we have compared their effectiveness at disrupting nuclear foci in a cell-based assay, which uses high-content imaging to 6363glier63 the number, size and intensity of foci. Immortalized DM fibroblast lines and DM myoblasts were treated with different CMOs including DNAs, LNAs, PNAs, morpholinos and 2 O-methyl modified oligos complementary to the CUG and CCUG repeat RNAs.
We find that two types of CMO; PNAs, and 2 O-methyl modified oligos are readily taken up by DM cells at nanomolar concentration, in the absence of transfection reagents, whereas the other CMOs are less effective at significantly higher concentrations. The CMOs have been further assessed in a series of assays to examine their effect on the nuclear to cytoplasmic location of the mutant transcript, muscleblind distribution and alternative splicing of various DM-related transcripts. We have developed a zebrafish model with DM-associated repeat expansion transcripts and have compared the effectiveness of the CMOs to correct the defective phenotype in this model.
[1]Mulders *et al* (2009) PNAS 106, 13915–13920,
[2]Wheeler *et al* (2009) Science 325, 336 – 339.

Poster 76

The LGMD diagnostic and advisory service in Newcastle, a multidisciplinary approach.

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The National Specialist Commissioning Team (NSCT) Diagnostic and Advisory Service for Rare Neuromuscular Disorders was established in 2001 as a consortium of four Centres in the UK to advance diagnostics in such diseases. Newcastle is the national referral centre for the diagnosis of limb girdle muscular dystrophies (LGMDs), providing a free service for patients referred from England, Scotland and Wales. LGMDs are caused by at least eight genes with dominant inheritance and seventeen genes with a recessive inheritance. Reaching a final diagnosis requires identifying the defective gene. Patients' diagnosis, however, is challenging due to the considerable overlap in clinical presentation not only among different LGMD subtypes but also with other forms of muscular dystrophy. Protein analysis of muscle biopsies is a useful test for ruling in or out a significant number of these pathologies. In Newcastle we offer multidisciplinary assessment of LGMDs with highly specialist clinicians and physiotherapists and comprehensive laboratory diagnostics provided at both the protein and DNA levels. An active research and development programme is in place to further characterise newly discovered disease-causing genes and their phenotypical spectrum. Our approach is beneficial to patients as the specialised diagnostic expertise is concentrated in one centre leading to more effective diagnoses and management.

Poster 77

Bethlem myopathy presenting as a limb girdle muscular dystrophy

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We report on a 49-year old man who presented at the age of 34 years with progressive proximal lower limb muscle weakness. Up until his early thirties he was fit and sporty. On clinical examination he showed symmetrical scapular winging, significant calf hypertrophy with medial gastrocnemius wasting, bilateral foot drop and knee hyperextension. His Achilles tendons were tight but he did not have any other contractures. His spine was straight and mobile and there were no significant skin changes. No cardiac involvement was detected and his respiratory function was satisfactory (FVC 3.29 litres or 78% of the predicted value). His creatine kinase levels were persistently raised above 1500 IU/L and an EMG showed myopathic changes. Muscle histopathology showed features of a muscular dystrophy with fiber size variation, internal nuclei, nuclear bags and ring fibers. Protein expression was normal on immunohistochemistry and Western blot. Based on the suggestive clinical and histopathological picture mutation analysis of the autosomal recessive limb girdle muscular dystrophy genes anoctamin 5 and dysferlin was performed but turned out to be negative. A diagnostic muscle MRI of the pelvic girdle and leg muscles showed a pattern of muscle involvement very suggestive of a collagen VI-related disorder, with the periphery of the muscles, especially the vasti, being more affected than the central part (concentric atrophy). The diagnosis of Bethlem myopathy was genetically confirmed by detection of a mutation in exon 32 of the gene COL6A3 that has been identified as disease-causing before. This case study illustrates that the broad phenotypic spectrum of collagen VI-related disorders ranges from Ullrich congenital muscular dystrophy and Bethlem myopathy to limb girdle muscular dystrophies (LGMDs), and that there can be considerable phenotypic overlap between collagen VI-related disorders and the autosomal recessive LGMDs.

Poster 78

Autosomal recessive desminopathy with desmin-null mutations presenting in childhood

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We report on two siblings from healthy, non-consanguineous parents presenting with progressive generalised proximal more than distal muscle weakness in legs and arms from the age of 3 years, facial weakness and ptosis, reduced upward gaze, scapular winging, ankle contractures, mild calf hypertrophy, respiratory involvement and swallowing difficulties. No cardiac involvement was detected so far. Creatine kinase levels were raised up to 800 and 1000 IU/L. A muscle MRI of the thigh showed more severe involvement of the posterior compartment with relative sparing of the sartorius and gracilis muscles. Muscle histopathology showed myopathic features with atrophic fibers, nuclear bags, internal nuclei and type 1 fibre predominance with remarkably complete absence of desmin labelling which was confirmed by Western blot. Mutation analysis revealed two compound heterozygous mutations in exon 1 of desmin gene resulting in premature stop codons. This is the first report of autosomal recessive desmin null-mutations resulting in complete absence of desmin on muscle biopsy immunohistochemistry and Western blot.

Poster 79

Getting to the Core of the Matter: Cores as a Common Muscle Pathology Finding in the Collagen VI- Related Myopathies

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Objective: The clinical phenotypes associated with a deficiency of collagen VI in the extracellular matrix of muscle are collectively termed the collagen VI-related myopathies. The interpretation of muscle biopsies in the collagen VI-related myopathies can be challenging, since specific morphological findings are not present, the pathology is often not overtly dystrophic and the immunohistochemical studies of collagen VI can be difficult to interpret in cases in which the basal lamina defect is subtle. The muscle histopathology finding of cores is classically associated with the core myopathies but can be seen in other muscle conditions, including collagen VI-related myopathies.
Method: A retrospective review of muscle biopsies in patients with molecularly confirmed collagen VI-related myopathies.
Results: In 20 molecularly confirmed collagen VI-related myopathy patients, the muscle biopsies of 12 patients had evidence of cores. All muscle biopsies had fibres positive for neonatal myosin.
Conclusions: This study highlights that cores are a common histopathology finding in muscle biopsies of molecularly confirmed collagen VI-related myopathy patients. It is, therefore, important to consider collagen VI-related myopathies when cores are seen on muscle biopsy and the clinical history, examination findings and/or muscle imaging findings are suggestive of this congenital muscular dystrophy subtype. The presence of large and small fibres positive for neonatal myosin provides a very useful marker to distinguish collagen VI-related myopathies from core myopathies and other congenital myopathies. These histopathology findings improve the frequently challenging diagnostic pathway for the collagen VI-related myopathies and potentially offer insights into disease mechanisms resulting from collagen VI deficiency.

Poster 80

Identification of novel variants in patients with non-collagen VI Bethlem myopathy by the emerging technology of exomic sequencing

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Mutations in COL6A1, COL6A2 and COL6A3, the genes which encode the extracellular matrix component collagen VI, lead to Bethlem myopathy (BM), Ullrich's Congenital Muscular Dystrophy (UCMD) and myosclerosis myopathy. Clinical history and examination data, as well as DNA and, where available, skin and muscle biopsy sections were collected from over 100 individuals with a clinical phenotype suggestive of collagen VI-related muscle disease. Through our research efforts to

better diagnose BM patients, firstly SCAIP (single condition amplification/internal primer sequencing) and more recently using a two-step algorithm based on the immunofluorescent analysis of collagen VI in dermal fibroblast cells, we have found the causative mutation in approximately 50% of our cohort. Whilst cryptic mutations cannot be ruled out, we suggest that there is significant genetic heterogeneity in BM. Since the majority of this non-collagen VI BM cohort are single cases from small non-consanguineous families, traditional genetic approaches used to identify novel disease causing genes are difficult to apply. Exomic sequencing is a powerful new technique which aims to identify the genetic basis of rare monogenic diseases by the direct sequencing of all coding regions of the genome (the 'exome') in an affected individual. We have used this approach to generate a number of candidate genes and variants of uncertain pathogenicity in a subset of our non-collagen VI BM cohort. These variants are ranked according to a number of factors: 1) occurance in more than one of our cohort, 2) likely pathogenicity of variant based on *in silico* analysis, type of mutation and evolutionary conservation and 3) genes known to be expressed in muscle or have known dysfunction in muscle diseases. The best candidates based on the described hierarchy were evaluated in the remainder of the cohort by PCR and direct sequencing with the aim to uncover a novel causative gene for non-collagen VI BM.

Poster 81

Diagnosis by sequencing: correction of misdiagnosis from FSHD2 to LGMD2A by whole exome analysis

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We studied and validated facioscapulohumeral muscular dystrophy (FSHD) samples from patients without a D4Z4 contraction (FSHD2 or 'phenotypic FSHD'). For this, we developed non-radioactive protocols to test D4Z4 allele constitution and DNA methylation, and applied these to patient samples from the Coriell Institute Cell Repository. The D4Z4 sizing showed two related patients to have classic chromosome 4 contraction-dependent FSHD1. A third sample (GM17726) did not have a short chromosome 4 fragment, and had been assigned as non-4q FSHD (FSHD2). We tested D4Z4 haplotype and methylation for this individual but found both to be inconsistent with this diagnosis. Using exome sequencing, we identified two known pathogenic mutations in *CAPN3* (Arg490Gln and Thr184Argfs*36), indicating a case of LGMD2A rather than FSHD. Our study shows how a wrong diagnosis can easily be corrected by whole exome sequencing by constraining the variant analysis to candidate genes after the data have been generated. This new way of "diagnosis by sequencing" is likely to become commonplace in genetic diagnostic laboratories. We also publish a digoxigenin-labeled Southern protocol to test D4Z4 methylation. Our data supports hypomethylation as a good epigenetic predictor for FSHD2. The non-radioactive protocol will help to make this assay more accessible to clinical diagnostic laboratories and the wider FSHD research community.

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Poster 82

Compound Screening in Myotonic Dystrophy

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We have developed a cell-based assay to assess the presence, size, intensity and number of nuclear foci in DM cells and have used this as primary screening assay to identify suitable compounds as possible starting points for a drug development program. A pipeline of primary, secondary, and tertiary assays has been developed to establish the consequences of compound treatment on key aspects of DM pathophysiology. Previous work on this project has involved the screening of 3,000

compounds from an FDA approved known drugs collection and 10,000 compounds from a small molecule compound library. The primary screening assay employs an *in situ* hybridisation protocol on immortalized DM cell lines, with a fluorescently labeled probe against the repeat expansion sequence, analyzed via high-content imaging on a scanning plate reader. From this screening assay we have identified 4 hit compounds that successfully reduce foci number.
Following identification in the primary screening assay compounds have been subjected to detailed analysis comparing compound sensitivity to eliminate foci versus toxicity to cells. Secondary assays have also evaluated the minimal structural requirements of the hit compounds by structure activity relationship analysis. Compound derivatives have been processed through the *in situ* hybridisation assay to determine the minimal structural requirements for a reduction in foci number. The results from these experiments aim to direct subsequent rounds of compound synthesis to produce the optimum compound structure that maintains the ability to reduce foci number with low toxicity to the cells.
A subset of tertiary assays has assessed the downstream consequences of the compounds on key aspects of DM pathophysiology. These assays have been optimized previously and allow us to examine the cellular location of the repeat expansion transcript, alternative splicing patterns of specific transcript isoforms (SERCA1, INSR and MBNL2) and the cellular distribution of the MBNL protein, all known to be affected in DM. Each of these tests provide support, or otherwise, for the suitability of a compound as a therapy for DM.

Poster 83

Myofibrillar myopathies (MFM), valosin containing protein (VCP) and glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase (GNE) genes' mutations are not associated with sporadic inclusion body myositis (sIBM)

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Background: Myofibrillar myopathies (MFM), inclusion body myopathy with dementia and Paget disease of bone (IBMPFD) and glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase (GNE) myopathy are genetically determined myopathies that can mimic sporadic inclusion body myositis (sIBM), especially if the disease phenotype is incomplete (e.g. skeletal muscle involvement only), if there is no family history (either because a mutation arises in the germ line or because the disease in the parents was unrecognised) or if the histological features overlap with those of sIBM. It is therefore possible that some sIBM cases may be caused by undetected mutations in the IBMPFD, GNE myopathy and MFM causative genes.
Objective: To investigate the association of sIBM with mutations in the MFM, IBMPFD and GNE myopathy causative genes.
Methods: Twenty nine patients with pathologically or clinically defined sIBM were screened for mutations in the following genes: desmin (DES), myotilin (MYOT), crystallin alpha-B (CRYAB), valosin containing protein (VCP) and GNE.
Results: No pathogenic mutations in the DES, MYOT, CRYAB, VCP and GNE genes were detected in this group of patients with sIBM.
Conclusion: This study provides evidence that common mutations in the DES, MYOT, CRYAB, VCP and GNE genes are not associated with the development of sIBM. Our results support current clinical practice in patients with sIBM, which are not usually screened for mutations these genes, unless the history or biopsy findings suggests a genetically determined myopathy.

Poster 84

Investigating mitochondria in cell culture models of core myopathies

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Core myopathies are inherited disorders presenting with disabling muscle weakness in infancy, due to mutations in the skeletal muscle ryanodine receptor (RYR1). RYR1 regulates calcium release from the sarcoplasmic reticulum to initiate contraction. Apart from being responsible for muscle contraction, calcium signals regulate mitochondrial bioenergetic function, movement, biogenesis, and free radical generation. The cause of the muscular weakness in core myopathies is unknown. We hypothesise that altered calcium signalling in core myopathy patients negatively impacts upon mitochondrial function, thus contributing to the muscle weakness. In order to assess this, we created cellular models of core myopathies, consisting of myoblast cultures established from patients and in some cases immortalized. To mimic the effect of nonsense mutations in some patients, we treated immortalized control cells with *RYR1* siRNA oligonucleotides. In differentiated myotubes in these models we measured the mitochondrial membrane potential by confocal microscopy and found mutation-dependent differences in patients compared to controls. This suggests that mitochondrial function is compromised, although further studies are needed to pinpoint whether the alteration is due to substrate availability or to mitochondrial ATP-synthase malfunctioning. In non-immortalised cell lines, activity of complex I of the respiratory chain is increased in patients compared to controls. Mitochondrial DNA copy number, measured by quantitative PCR, appears to vary depending on the *RYR1* mutation type. This study suggests that mitochondria have a major impact in the pathogenesis of core myopathies and forms the basis of future studies into the mechanism.

Poster 85

Colchicine myopathy: Pathological analysis of a case with novel findings

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Introduction: Long term use of colchicine, a microtubule polymerisation inhibitor can induce a toxic myopathy and axonal neuropathy in humans.
Case Report: A 76 year old man, with a complicated past medical history including inflammatory arthropathy secondary to gout and possibly rheumatoid arthritis, mild renal impairment and COPD was admitted with an acute respiratory infection and worsening mobility 2 weeks after minor surgery. His medications on admission included colchicine 500mg BD, atorvastatin 40mg OD and prednisolone. It transpired that his mobility had been worsening over 3 years. Examination and investigations revealed proximal limb weakness, reduced reflexes, minimal sensory signs, raised creatine kinase and an axonal neuropathy. The colchicine he had taken for many years was stopped leading to discernible functional improvement. A right sural nerve biopsy showed an axonal neuropathy and small vessel vasculitis. A right quadriceps biopsy showed florid vacuolar myopathy affecting both fibre types. Larger vacuoles were often empty and smaller vacuoles often rimmed, with acid phosphatase activity. Prominent subsarcolemmal and internal deposits of dark staining material stained with NADH, SERCA1 and 2, and co-localised with dynein, alpha-tubulin, dysferlin and phalloidin. Aggregates of desmin, myotilin, p62 and alpha B crystallin were noted. C5b-9 and HLA lined the sarcolemma of several fibres. Electron microscopy showed numerous autophagic vacuoles, myofibrillar disarray and lysis, sarcotubular proliferation, thin filament accumulation and novel inclusions containing tightly packed alternating electron-dense and electron-lucent internal profiles.
Conclusions: The pathology in our case highlights the effect of colchicine on microtubules, similar to previously documented cases. The prominent protein aggregation may point to alternate pathomechanisms of colchicine toxicity. The precise nature of the novel ultrastructural inclusions is not known.

Poster 86

Frequency and circumstances of falls in people with Inclusion Body Myositis

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Falls are commonly reported by people with neuromuscular disorders. A recent survey of people with Charcot Marie Tooth disease showed that 89% of respondents have fallen and 30% of respondents fall at least once a month. It is well established that falls in the elderly population can cause serious injury and lead to reduced mobility through fear of falling or by injury sustained when falling. Inclusion body myositis (IBM) is the most common muscle disease diagnosed in the over 50s and clinical reporting suggests falls are more frequent than would be expected in this patient group than in an age matched healthy population.
This study aimed to survey people with IBM, to ascertain how often they fall or nearly fall and the circumstances that may have contributed to the fall.
61 people with IBM were sent questionnaires and 39 responded (64%). Falls were reported by 97% of respondents with 28% of respondents falling at least once per month. 69% of respondents fell at least once in the last three months prior to completing the questionnaire. Only 10% of respondents had not nearly fallen and of those that had, 64% nearly fell at least once a month.
The most common locations for falls were outdoors (43%) and in the home (41%). Walking was the most common activity being carried out when falls occurred (56%) and of all falls the most likely commonly blamed cause was leg muscle weakness (38%) with other likely causes being attributed to loss of balance (18%) and tripping (16%).
Minor injuries were the most common outcome (55%) and many falls resulted in no injury (32%), but moderate and major injuries did occur (6%).
We conclude that falling is a common and clinically important problem in patients with IBM. Health care professionals who manage patients with IBM should routinely address falls. Understanding why IBM patients fall will help in developing tailored interventions that can effectively safeguard IBM patients from falling.

Poster 87

Cytoplasmic Dynein Heavy Chain 1 causes autosomal recessive congenital distal SMA

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A tail domain mutation in Cytoplasmic Dynein Heavy Chain 1 (*DYNC1H1*) that causes a rare form of dominantly inherited SMA with early childhood onset of weakness and disproportionate involvement of the legs (SMA-LED) has been reported.
We report a case with congenital SMA caused by recessive *DYNC1H1* mutations. A ten year old girl presented with floppiness and joints contractures at birth, followed by motor developmental and speech delay and learning difficulties. Examination at nine years showed trunk adiposity and thinning of lower legs. Hip adductors were subgravity while hip extensors and trunk flexors were MRC 3. She could walk with gaiters and transfer herself from wheelchair. Serum CK was normal. Sensory and motor NCV in lower limbs were normal while EMG showed severe chronic denervation. A quadriceps biopsy showed myopathic features and small core-like lesions. Legs muscle-MRI showed symmetrical diffuse fatty replacement with sparing of the extensor compartment. Sanger sequencing showed a homozygous mutation in tail domain of *DYNC1H1* (R399G). Both parents were heterozygous for the same variant and a copy number variation at the *DYNC1H1* was ruled out by SNP array. Father was also mildly affected with neurogenic EMG changes.
In conclusion we describe a girl affected by a severe form of congenital SMA affecting predominantly the lower limbs. She was homozygous for a previously unreported *DYNC1H1* variant affecting the tail domain. Mutations in DYNC1H1 may to be a common cause for congenital SMA-LED; further functional studies are needed to understand the pathogenesis of this motor neuron disease.
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Poster 88

Nebulin mutations in a childhood onset distal myopathy with rods and cores uncovered by next generation sequencing

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Mutations in the nebulin gene (*NEB*) are a common cause of autosomal recessive nemaline myopathy (NM), characterised by the presence of rods. In a single adult case with *NEB* mutations, cores were also present. A few adult patients with distal involvement showing neither rods nor cores have also been described

We report a case with childhood onset recessive distal myopathy with cores and rods caused by recessive *NEB* mutations. A seven year old boy presented to us with a four year history of gross motor difficulties, such as running and jumping, after normal motor milestones, nasal speech, and recurrent chest infections. On examination he had axial muscle weakness, with respiratory insufficiency requiring BIPAP, scoliosis and rigidity of the spine, and also significant distal weakness with bilateral footdrop. Serum CK was normal. The muscle biopsy showed the presence of rods and cores. Mutations in the *RYR1 and KBTBD13* genes were excluded. Muscle imaging of his legs showed a distal involvement in the medial and anterior compartment. Molecular analysis of *NEB* showed a heterozygous exon 55 deletion while next generation sequencing which also studied 50 additional genes, identified another heterozygous mutation c.24267_24270dup (p.Val8091fs) in *NEB* exon 172. This case is of interest because of the unusual combination of early onset distal and axial muscle weakness together with rods and cores, illustrating that *NEB* can be a cause of childhood onset distal nemaline myopathy with cores. Furthermore, next generation sequencing is a valuable tool for the molecular diagnosis of congenital myopathies.

Poster 89

Using Whole Exome Sequencing to Identify the Mutation Causing Oculopharyngodistal Myopathy

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Background: Oculopharyngodistal myopathy (OPDM) is a rare adult onset hereditary muscle disease with putative autosomal dominant and recessive inheritance. Patients commonly show ptosis as an initial symptom followed by distal weakness and swallowing difficulties. A significant number of patients develop respiratory muscle weakness but none exhibit a cardiomyopathy. Muscle histology reveal chronic myopathic changes with rimmed vacuoles. The genetic defect causing OPDM has not been determined as yet.

Methods: Two patients from a large Turkish family underwent whole exome capture sequencing. Genome wide linkage as well as haplotyping for regions of interest was carried out using SNPs and microsatellites markers. Sanger sequencing and restriction endonucleases were used to confirm novel variants found in the exome sequencing.

Results:We received a LOD-score of almost 3 for a dominant model in a parametric linkage analysis on a certain region on chromosome 10. Two candidate variants, identified through exome sequencing, were positioned in the locus of interest and segregated well with the disease in some of the families. We are in the process of validating these variants using haplotyping and direct sequencing in other individuals.

Poster 90

Cardiac MRI in LGMD2I patients

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Limb Girdle muscular dystrophy 2I is known to be associated with cardiomyopathy and regular cardiac surveillance is required in the care of these patients. Cardiac MRI has been reported as being more sensitive in detecting changes earlier than conventional methods. Ten patients, 7 male patients and 3 female patients, with a mean age of 47 years, ranging from 22 – 65 years and all with a diagnosis of LGMD2I underwent cardiac MRI as part of a larger longitudinal skeletal MRI study.
The most significant functional impairments found by cine-MRI in the LGMD2I patients were reduced ejection fractions (mean 47% vs 58% in controls, $p = 0.017$) and reduced stroke volume (61ml vs 81ml, $p = 0.038$)
Peak cardiac torsion was significantly reduced in the LGMD2I patients compared to the controls (mean 3.9^{o} vs 6.4^{o}, $p = 0.038$), while there was no significant change in peak circumferential strain (whole wall) achieved (16.4% vs 18.3%, ns). Impairments in peak cardiac torsion and the torsion to endocardial strain ratio correlated strongly with impairment in ejection fraction ($r = 0.93$, $p < 0.001$ and $r = 0.88$, $p < 0.004$ respectively). The ratio of PCr/ATP was significantly reduced in the LGMD2I patients compared with the control group as a whole (mean 1.50 vs 1.94, $p < 0.0005$)
This data gives an insight into the pathogenesis of cardiac dysfunction, with early reduction in myocardial "7171glier71" which worsens with age, and early reductions in cardiac torsion and ejection fraction.

Poster 91
A Morpholino Antisense Oligonucleotide Rescues Type I and Type III SMA Mice
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The majority of patients affected by Spinal Muscular Atrophy (SMA) are due to homozygous deletions of Survival Motor Neuron gene 1 (*SMN1*) which leads to the absence of functional SMN protein, and results in motor neuron degeneration and muscle weakness. A second gene, *SMN2*, encodes a related protein, but a single nucleotide difference from *SMN1* prevents efficient splicing of exon 7, leading to a truncated transcript and less functional and unstable protein. Modification of the endogenous *SMN2* pre-mRNA splicing is a promising therapeutic strategy for SMA. Morpholino antisense oligonucleotides have been successfully used in Duchenne muscular dystrophy clinical trials and showed satisfactory safety in preclinical and clinical studies. In this study, we have developed a new morpholino antisense sequence which targets the intronic splicing silencer in intron 7 of SMN genes. *In vitro* data shows this antisense oligonucleotide induces exon7 inclusion in SMA fibroblasts in 100% of the transcript. *In vivo* validation shows this morpholino antisense oligonucleotide rescues both type I and type III SMA mice by systemic delivery in neonatal mice. While the therapeutic window in severe type I SMA mice is between post neonatal day 0 to day 3, we show here that Injection of the morpholino antisense oligonucleotide at post neonatal day 14 to type III mice still has therapeutic efficacy in delaying tail necrosis, which suggests a wider therapeutic window in the less severe type of SMA mice and that rescue of SMN expression in peripheral tissues still has an effect on SMA phenotype in these mice. Our study suggests this morpholino antisense oligonucleotide is an encouraging candidate for future clinical trials in SMA.

Poster 92
RyR1 deficiency in congenital myopathies disrupts excitation-contraction coupling and induces the second calcium release system via IP3R
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In skeletal muscle, sarcoplasmic reticulum calcium release is triggered by direct activation of the ryanodine receptor (RyR1), situated on the terminal cisternae of the sarcoplasmic reticulum, by the voltage-gated dihydropyridine receptor (DHPR) located on the transverse tubules. Inositol-1,4,5-triphosphate receptor (IP3R) is the major calcium release channel in the endoplasmic reticulum of non-skeletal muscle required for pharmacomechanical coupling. In this study, we present evidence that the distribution and consequently the physiological interaction of these proteins are disrupted in patients with *RYR1*-related congenital myopathies that are caused by primary RyR1 deficiency and secondarily altered DHPR expression. By creating a cellular RyR1 knock-down model using immortalized myoblasts treated with RyR1 siRNA, we further confirmed this observation *in vitro*. Unexpectedly, we also found up-regulation of IP3R in RyR1 SiRNA treated immortalized muscle cells, at both mRNA and protein levels. Further experiments on skeletal muscle samples from patients that had RyR1 deficiency caused by recessive *RYR1* genotypes, confirmed consistent up-regulation of mRNAs of all three types of ITPR genes as well as up-regulation of IP3R proteins. In addition, RyR1 knock-down in cultured human myotubes affected the spatial expression of IP3R, with a shift from the nuclear envelope to the rest of the sarcoplasmic reticulum. Thus our results indicate that a second calcium release system is up-regulated in RyR1 deficiency.

MRI in Neuromuscular Disease

Poster 93

Manganese enhanced MRI as a useful *in vivo* outcome measure in assessing skeletal muscle calcium uptake in mouse models of muscular dystrophy

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It is well documented that intracellular calcium levels are elevated in DMD and in the mdx mouse model. We have previously used manganese enhanced MRI (MEMRI) a technique which utilizes the T1 contrast enhancing properties of Mn^{2+} as an *in vivo* marker for Ca^{2+} uptake in mdx and sgcd-/- myocardium. Here we have measured Mn^{2+} uptake in the pectoral and triceps, the most consistently visible skeletal muscles in the cardiac acquired scans.
Results show Mn^{2+} uptake to be elevated in mdx mice and further enhanced in sgcd-/- when compared to BL10 controls. Initial results in dysferlin mice (n=3) show similar uptake to BL10 in triceps though reduced uptake in pectoral muscles. Older mice in all groups have lower uptake than younger mice within the same group and diltiazem (a non DHP L-type calcium channel blocker) has differential effects in mdx and sgcd-/- mice.
This study provides an *in vivo* insight into calcium uptake by skeletal muscle and the technique may provide a useful outcome measure when looking at the effectiveness of therapeutic agents in muscle.

Poster 94

MRI quantification of abnormal muscle water distribution in chronic neuromuscular diseases: a sensitive biomarker

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Background: Quantitative muscle MRI in neuromuscular diseases can measure abnormal muscle water distribution through measurement of T2 relaxation times and the magnetisation transfer (MT) ratio, which may precede fatty infiltration and potentially provide a sensitive biomarker.

Methods: 20 patients with Charcot-Marie-Tooth disease type 1A (CMT1A), 20 patients with inclusion body myositis (IBM) and 27 matched controls underwent 3T MRI including short-tau inversion recovery (STIR), 3-point Dixon fat quantification, pseudo-T2-mapping and MT sequences. MRI analysis was performed blinded to diagnosis.
Results: There was qualitative STIR hyperintensity in more than half of lower limb muscles in IBM patients and in 31% of calf muscles in CMT1A patients. T2-times were significantly increased and MT ratio significantly reduced in thigh and calf muscles of IBM patients and calf muscles of CMT patients compared with controls. These differences remained significant ($p<0.001$) when only muscles with normal fat levels (<5%) on Dixon imaging were included, displaying T2 times of (mean ± s.d. milliseconds): thigh – IBM 50.2±8.1, CMT1A 43.4±5.3, control 43.1±5.0; calf – IBM 46.6±7.3, CMT1A 42.6±5.0, control 41.0±3.6; and MT ratios of (mean ± s.d. percentage units): thigh – IBM 29.4±2.6, CMT1A 31.7±2.0, control 31.9±1.6; calf – IBM 30.4±2.4, CMT1A 31.4±1.7, control 32.1±1.3.
Conclusions: We demonstrated abnormal muscle water distribution in calf muscles in CMT1A and calf and thigh muscles in IBM using qualitative STIR and pseudo-quantitative T2 and MT sequences. The differences were greater in IBM patients than in CMT1A patients. The changes were evident in muscles with normal fat fraction, indicating these biomarkers are sensitive to early disease changes.

Poster 95
Novel Muscle Fat-Fraction MRI Metrics for Quantifying Neuromuscular Pathology
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Introduction Muscle fat infiltration in NM disease is often assessed by visual classification of appearance on T1w MRI. Increasingly, fat quantification methods are being used to map the muscle fat-fraction (FF) with results usually reported as mean value over a region of interest (ROI). More sophisticated metrics, such as those describing FF histogram features, may capture subtle characteristics of infiltrating fat distributions not reflected in simple reductive measures such as mean FF.
Methods Twenty-six volunteers, 18 inclusion body myositis patients and 18 CMT1a patients underwent 3T lower-limb MRI. T1w MRI allowed visual classification of fatty infiltration in each muscle using the 'Mercuri' 6-point scale. Corresponding 3-point Dixon FF maps were generated. ROIs were defined on 6 calf muscles in each limb and a histogram analysis conducted for each muscle, including calculation of the first 4 moments of the FF distribution, i.e. the mean, standard deviation, skew and kurtosis.
Results The FF mean and standard deviation both correlated positively with visual grade as expected ($\rho=0.89$ and 0.81, $p<0.01$). The FF distribution skew and kurtosis correlated negatively with visual grade ($\rho=-0.72$ and $\rho=-0.68$, $p<0.01$) in all muscles except tibialis posterior.
Discussion These statistical associations support the hypothesis that more sophisticated FF histogram-based parameters will be of value in quantifying muscular pathology. Future work will determine if these are sensitive to the early temporal evolution of fatty changes, or can identify subtly disease-affected individuals suitable for inclusion in clinical trials with increased likelihood of treatment response.

Neuromuscular Databases for Translational Research and Clinical Trials

Poster 96
Moving Forward with TACT: 2 years on
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The TREAT-NMD Advisory Committee for Therapeutics (TACT) was established in 2009. The Chair works closely with a core group, and 45 additional international multidisciplinary experts, each with

specific expertise in one or more areas of drug development to provide the neuromuscular community (clinicians, researchers, patient advocacy groups and industry) with independent and objective guidance on advancing new therapies, whether novel or repurposed, for neuromuscular diseases. TACT is supported by the TREAT-NMD secretariat and Patient representation for the disease programs evaluated is included in the TACT reviews.
The goal of each review is to position the potential therapy along a realistic and well informed pathway to clinical trials, and eventual registration, by evaluating the supporting preclinical data and all other critical drug development considerations that are necessary for objective decision-making and for the design and conduct of studies that generate meaningful data and have the potential to be funded longer term.
To date TACT have held four meetings and have reviewed a total of eleven program applications from both academic investigators and industry. Feedback so far shows that TACT has generated recommendations that have helped investigators evaluate their potential compounds and consider the development program in a methodical fashion with clear go-no go decisions and with optimal use of funding and resources.
The next TACT meeting will be held on 28th-29th April 2012 in Arlington, Virginia, USA. The subsequent meeting will be held in November 2012 in Europe. Investigators interested in submitting an application for review should contact Emma.Heslop@ncl.ac.uk.

Poster 97
Clinical Research Activity in Newcastle MRC Centre
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The Newcastle MRC Neuromuscular team encompasses a number of specialists involved in the diagnosis, treatment, management and research into a broad spectrum of neuromuscular and mitochondrial diseases, ranging from Duchenne muscular dystrophy, congenital myopathies and limb girdle muscular dystrophies to mitochondrial cytopathies and inherited neuropathies. The team aims to use information gathered from transitional research to offer patients suffering from genetic and acquired neuromuscular diseases the opportunity to take part in studies and clinical trials, which may lead to new treatments and improve the quality of life for all patients and their families. The clinical research team includes clinicians, clinical research associates, physiotherapists, research nurses, clinical trial coordinators and PhD students working together on a number of studies in adult and paediatric patients. Current and pending projects include drug (8) and exercise intervention studies (7), transitional research (9), natural history (3), registries (2), BioBanks (2), cohorts (1) and clinical outcome studies (3). The team is active in the conception and design of local, national and international commercial and academic studies. The coordination team is responsible for obtaining Ethical and Research and Development approval and study management throughout the whole process. Every member of the clinical research team is instrumental in conducting research in line with Good Clinical Practice (GCP) which is facilitated by the coordination team to produce the highest professional level of neuromuscular clinical research in Newcastle.

Poster 98
New patient registries for Myotonic dystrophy and Facioscapulohumeral muscular dystrophy in the United Kingdom
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Patient registries for rare diseases have proved invaluable over the past few years as they have been instrumental in clinical trial planning and recruitment, as well as being useful for the

dissemination of standards of care. Two new registries for Myotonic dystrophy (DM) and Facioscapulohumeral muscular dystrophy (FSH) are under development within the UK.
The DM registry, based at Newcastle University, has been developed in collaboration with the TREAT-NMD Alliance and two UK patient organisations, the Myotonic Dystrophy Support Group (MDSG) and the Muscular Dystrophy Campaign (MDC). The registry uses an agreed core data set that includes several mandatory data items that must be collected by the registry, and is a combination of self- and clinician-report. The registry has recently received full ethics approval and will be launched soon. An oversight committee has also been established.
A core date set for the FSH registry has already been agreed and will be applied to the planned FSH registry once ethical approval has been received. A solution to the registry software is being procured and will be closely based on the DM registry design.
A global registry for Myotonic dystrophy is under construction and there are several national DM registries already running in many countries which will feed into the global registry once it is up and running. TREAT-NMD Alliance is working in close collaboration with both the DM and FSH registries to ensure that the neuromuscular community is prepared for clinical trials with emerging new therapies in these diseases.

Poster 99
MRC NMD Centre Biobank: An overview
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A lack of access to biomaterials from patients with neuromuscular disorders (NMD) has hampered research into and the development of potential therapies. The MRC Centre for Neuromuscular Diseases Biobank is a unique repository of human biomaterial aimed at facilitating research of NMD by providing a continuous availability of high quality human biomaterial including DNA, muscle and skin cell lines and also body fluids such as urine, blood, plasma and serum to the scientific community within an ethical framework. The Biobank has been established in close collaboration at Newcastle University and UCL London under the responsibility of Professors Hanns Lochmüller and Francesco Muntoni. The Biobank has been actively collecting human biopsy samples since June 2008 from both sites in London and Newcastle using the large neuromuscular patient population seen at both MRC Centers. Biopsy samples are predominantly collected from patients with a confirmed or suspected diagnosis of muscular dystrophy. Blood samples from patients with a confirmed diagnosis of muscular dystrophies have been collected, stored and provided to UK and EU partners as part of the EU-funded BIO-NMD project. Within the first 4 years of establishment of the centre both Biobank's have surpassed the milestones and benefited a large number of basic, translational research and research projects, have supported PhD students and high-profile publications and integrated successfully in UK and international networks. Moreover, the Biobank was indispensable in attracting significant subsequent funding from national (MRC and Wellcome Trust) and European (EC, 7th framework program) agencies.

Poster 100
CARE-NMD: Improving care for Duchenne Muscular Dystrophy
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CARE-NMD is a three-year EC-funded project to evaluate current treatment of Duchenne muscular dystrophy (DMD), the most common childhood muscular dystrophy, and to implement international consensus care standards through a "reference network" of care centres in seven European countries.
Although expert care centres for neuromuscular disease exist in most European countries, many patients still do not receive treatment in line with best practice, particularly in Eastern Europe where there is a of lack information and access to diagnostic and care expertise. The publication of

international consensus guidelines for DMD treatment in *The Lancet Neurology* in 2010 has provided an opportunity to address health inequalities both within and between European countries.
The project is currently assessing the level of care available to those living with DMD, as well as their perceived quality of life, by means of questionnaires distributed via the national patient registries in each participating country. As of January 2012, almost 1000 responses have been received. In addition, a survey of clinicians is currently being prepared – to be distributed in early 2012 – to determine the availability of best-practice care for DMD.
The results of these surveys will be used to identify gaps in care provision, which can then be addressed through specialised training events organised by each national partner. These sessions will be tailored to local professional training needs. The results of the surveys will also be fed back to clinicians and patients. Through the creation and development of the reference network of care centres, the CARE-NMD aims to embed best practice care for DMD in national healthcare systems.

Poster 101

The International Dysferlinopathy Registry and An International Clinical Outcome Study for Dysferlinopathy

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A registry is being developed specifically for patients worldwide affected with a dysferlinopathy, including LGMD2B (Limb Girdle Muscular Dystrophy type 2B) and Miyoshi myopathy among all other clinical presentations. Patients are eligible to register if their clinical dysferlinopathy diagnosis has been confirmed by a genetic test with at least one pathogenic mutation in the dysferlin gene.
This International Dysferlinopathy Registry (IDR) collects the patients' exact genetic defects, key medical data about their disease including protein levels and clinical information, as well as personal details. This is aimed at giving patients a better opportunity to participate in research studies/ clinical trials (such as the Dysferlin Clinical Outcome study) and obtain the best possible care, and at providing patients with information about their condition.
Patients register by self-report, giving informed consent. Mutational data and protein levels are provided by the patients' doctors, upon patient and doctor consent. Third parties can request anonymised medical data from the registry and – subject to approval – use the obtained information for research, study/trial planning or planning, or patient recruitment.
The International Clinical Outcome Study for Dysferlinopathy will use the IDR to help with patient recruitment into the study. Study participants need to register with the IDR and the data collected during the study assessments will be linked to the participant's IDR entry. This study is endorsed and funded by the Jain Foundation and will take place over 4 years (1yr recruitment & 3yrs assessments) in 14 centres in Europe, the USA, Japan and Australia.
At least 150 genetically confirmed dysferlinopathy patients will be recruited (100 ambulant, 50 non-ambulant) who will be assessed at 6 visits over 3 years at physiotherapy, medical and MRI/MRS assessments. MRI will be performed in all patients and MRS in a subset. Biological samples from blood and skin for storage in a BioBank – where the samples will be accessible for approved research – will be collected upon separate informed patient consent.
The aim of the study is to characterise the natural progression and pathophysiology of dysferlinopathies, to delineate clinical outcome measures appropriate for future clinical trials, as well as to discover biomarkers that can be used to track disease severity and progression. Patient pre-screening/ recruitment is envisaged to start in spring/summer 2012. Travel funds are available for the study participants to attend the assessment visits.

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.

MRC Centre CTIMPs Set-up Phase trials

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 characterised 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, multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy in genetically diagnosed 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, placebo-controlled Phase III trial of combined ACE inhibitor 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. For more information about the study please contact the trial coordinator on 020 7905 2639.

A Pilot Study of Valproate Sodium for McArdle Disease

Status: Set-up phase
Sponsor: UCL
Planned start date: 2011
Funder: Muscular Dystrophy campaign
PI: Prof. Ros Quinlivan
Recruitment target: 15

McArdle disease (Glycogen storage disease type V, GSDV) is an inherited metabolic disorder of skeletal muscle. Affected patients are unable to produce lactate during ischaemic exercise [McArdle 1951] because they have a congenital absence of the enzyme muscle glycogen phosphorylase, which is essential for glycogen metabolism [Mommaerts 1959, Schmidt and Mahler 1959]. The condition is caused by homozygous or compound heterozygous mutations in the muscle glycogen phosphorylase gene *(PYGM)* located at chromosome 11q13 [Beynon 2002]. This enzyme deficiency results in the inability to 78uglieri muscle glycogen stores that are normally required for energy during anaerobic metabolism. In affected people, symptoms of fatigue and cramp occur within minutes of initiating any activity and during strenuous activity such as lifting heavy weights or walking uphill, if the activity is continued despite severe cramping, a contracture occurs which leads to muscle damage (rhabdomyolysis), myoglobinuria and, when severe, acute renal failure.

Currently, there is no satisfactory treatment that can be recommended for the condition [Quinlivan 2008]. Taking glucose prior to exercise may alleviate muscle symptoms by inducing a second 'second wind', but this is not a good strategy for daily living as it may result in significant weight gain [Vissing 2003]. There is limited evidence for subjective benefit from creatine supplementation in five out of nine subjects from a controlled trial [Vorgerd 2002], although this has not been confirmed in the clinic setting.

Although most people with McArdle disease have complete absence of skeletal muscle phosphorylase, there are a small minority of patients who possess splice site mutations that enable production of very small amounts (1-2%)of functional enzyme [Vissing]. These people have a milder phenotype with less severe symptoms, and functional exercise assessments have shown better exercise capacity than typical patients with the condition. Findings from these atypical individuals suggest potential therapeutic agents might only need to produce very small amounts of enzyme for significant functional improvement. Furthermore, finding a therapeutic agent to 'switch on' expression of the foetal isoenzyme may be a potential therapeutic strategy.

Sodium Valproate (Valproic acid) is one of a group of drugs known as histone deacetylase inhibitors (HDACIs) that can affect gene expression by acetylating lysine residues, which in turn has a direct effect on chromatin [Thiagalingam 2003]. There is some evidence from animal studies to suggest that sodium valproate can 'switch on' the foetal phosphorylase isoenzyme.

A recent clinical trial of the drug in McArdle sheep that were given sodium valproate for three months showed the presence of phosphorylase positive muscle fibres, in the absence of muscle necrosis and/or regeneration [Howell 2010].

The current proposes an open label uncontrolled pilot study to evaluate safety and efficacy of Sodium valproate (slow release) 20mg /kg once daily for six months. 15 subjects, adult male and post menopausal women attending specialist centres for McArdle disease will be recruited across three sites: London, Copenhagen and Dallas.

MRC Centre CTIMPs Open Trials

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: Open
Sponsor: TROPHOS
Funder: Association Francaise contre les Myopathies
Pis: Francesco Muntoni, Hanns Lochmuller, Helen Roper
Recruitment target (UK): 30; due for completion by 31st September 2013

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, randomised, 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 francaise 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 neuronic 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 able 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.

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:14; 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 or Gisela Barreto, Trials Coordinator at 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: Closed to Recruitment
Sponsor: University College London (UCL)
Funder: Arthritis Research UK and Myositis Support Group
PI: Prof. Hanna
Patients recruited: 12; 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@ucl.ac.uk or Gisela Barreto, Trials Coordinator at 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 lla, 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 recruit 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 MRC Centre 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: 39; 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. 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, 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

Patients recruited: 22, target: open-ended

Recent research suggested that lithium carbonate may be effective in lowing 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 take lithium or placebo, the level of lithium in the blood is monitored, and the dose of lithium (and placebo) adjusted as needed.

LiCALS Open Label Extension

Full title: LiCALS open label extension trial of lithium carbonate in amytrophic lateral sclerosis.

Status: Recruiting.
Sponsor: University College London Hospitals NHS Foundation Trust
Start date: March 2011
Funder: Motor Neurone Disease Association, and NIHR
UCL PI: Dr Richard Orrell
Patients recruited: 3 of 8 recruited

This is an open label extension study for those who have completed the double blind trial of lithium carbonate in ALS. The objective is to obtain further evidence of the safety of lithium carbonate in doses achieving levels of 0.4-0.8 mmol/l.

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

Full title: A Phase I, multi-center, randomised, 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
Patients recruited: 2, target: 2

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 leader 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.

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 diseases 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.

MRC Centre CTIMPs Completed 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 target 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, 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 MRC Centre for Neuromuscular Diseases. Paper published in Lancet Neurology 2010.

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: Completed
Sponsor: University College London (UCL)
Funder: Food and Drug Administration (FDA – USA)
PI: Prof. Hanna
Patients recruited: 14; 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. Fifteen participants have been enrolled in the UK at the MRC Centre. For information on the status of recruitment please contact Dr. Dipa Raja Rayan at d.rayan@ucl.ac.uk or Gisela Barreto, Trials Coordinator at gisela.barreto@uclh.nhs.uk.

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, 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 has 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 Briefly, analysis showed that, on average, patients treated with low-dose PTC124 experienced better outcomes on measures of efficacy than patients treated with high-dose PTC124 or placebo – this phenomenon is not unique 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).

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 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: Completed
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 ≥5 and ≤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 demonstrate 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 subject (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 published.

www.thelancet.com Published online July 25, 2011 DOI:10.1016/SO140-60756-3.

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 Immunosupressants

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 a important role in the disease process.

Patients will receive approximately 22 infusions including 11infusions 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 jennifer.spillane.09@ucl.ac.uk or Natalie James at 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 >10

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.rayan@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 >20

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 characterise 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.

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: 162; 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, 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 five-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 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@ucl.ac.uk.

MITOCHONDRIAL DISEASE COHORT

Status: Open to Recruitment
Sponsor: The University of Newcastle Upon Tyne
Funder: MRC
PI: Dr R McFarland, MG Hanna, DM Turnbull
patients recruited: 871; target 1000

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 characterised 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 1000 patients in total.

For information on the status of recruitment please contact Dr. Robert Pitceathly (London) r.pitceathly@ucl.ac.uk.

THE NATURAL HISTORY OF INCLUSION BODY MYOSITIS (IBM Net)

Status: Open to Recruitment
Sponsor: University College Hospitals
Funder: MDC
PI: Dr Matt Parton/Mike Hanna
Enrolled 164 Target 200

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 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@ucl.ac.uk.

24. PERIPHERAL NEUROPATHY OUTCOME MEASURES STANDARDISATION STUDY (PERINOMS)

Status: Open
Sponsor: Erasmus Medical Center
PI: Dr M Lunn
Patients recruited: 110; 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 Natural History 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

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

The aim is to collect standardised 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.rayan@ucl.ac.uk.

Exercise Studies

Set-up Phase

Aerobic training in Charcot-Marie-Tooth disease and Inclusion Body Myositis.

Status: In set-up
Sponsor: University College Hospitals
Funder: TBC
PI: Dr Gita Ramdharry

The specific objective of the present study is to investigate the effect of aerobic training in two common neuromuscular diseases (NMD): Charcot-Marie-Tooth disease (CMT) and Inclusion Body Myositis (IBM). These diseases result in progressive muscle wasting and substantial morbidity and disability. The effect of aerobic training on fitness levels, muscle strength and function will be systematically examined. This study will also monitor the safety, feasibility and impact on quality of life of this type of exercise training in these groups.

Sixty subjects, (30 from each disease group, aged between 18 and 75), will be recruited from the neuromuscular clinics at Queen Square. Both disease groups will be investigated concurrently with the same methods but will be viewed and analysed as separate studies. A crossover design will be used with training and control periods. The trial will span three years with each subject participating for a 34 week period. For the training intervention, participants will train in select local gyms and train on a bicycle ergometer.

The primary outcome measure for this study is maximum aerobic capacity during exercise testing. There will also be measures of muscle strength, body composition, and activity levels. In addition the study will investigate non-motoric effects of exercise such as mood, motivation, sleep and fatigue.

Full Title: Exploring the causes of falls and balance impairments in people with neuromuscular diseases

Status: In set-up
Sponsor: University College Hospitals
Funder: NIHR
PI: Dr Gita Ramdharry

Falls are commonly reported by people with neuromuscular disorders but to date there has been little formal investigation of this problem. Frequent falling increases the risk of injury and reduces mobility due to avoidance of activities perceived to increase the threat of falls.

The aim of this study is to ascertain falls risk from measurement of falls incidents, balance impairment and clinical presentation in people with different types of Charcot-Marie-Tooth (CMT), Distal Myopathy (DM) and Sensory Neuropathy (SN) with healthy controls. Measurements of static, anticipatory and reactive balance impairment and prospective falls events will be used to ascertain relationships with clinical presentation in people with different types of CMT, DM and SN. The three pathologies have been chosen for comparison as this will allow some discernment between the sensory and motor contributions to falls.

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

Collaboration site MRC Centre London (Hanna)
Patients recruited: 6 (5 Newcastle, 1 London)

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 are:
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 Dr Robert Pitceathly at 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 divided into two groups. They will receive either; exercise 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/m2; 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.

PHYSICAL ACTIVITY AND INCLUSION BODY MYOSITIS

Status: Recruiting
Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust
Funder: MRC
PI: Dr M Trenell
Collaborating site MRC Centre London
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.

EXERCISE AND SARCOPENIA

Status: Recruiting
Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust Funder: MRC
PI: Prof DM Turnbull
Collaborating site MRC Centre London
Patients recruited: 0; target: 36

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 7^{th} decade of life
6. To assess the 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.

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 target: 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, 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.

Data being analysed for publication.

Imaging Studies

Set-Up Phase

Full Title: Evaluation and Optimisation of Muscle Imaging Biomarkers in Support of Non-ambulant Duchenne Muscular Dystrophy Studies

Sponsor: Great Ormond Street Hospital / Institute of Child Health
Funder: GSK
PI: Prof Francesco Muntoni

Duchenne muscular dystrophy (DMD) is the most frequent inheritable lethal childhood disease. In the recent years, there have been promising advances for new potential genetic treatments (including the development of exon skipping with anti-sense oligomers producing dystrophin restoration). The current way to assess successful outcomes in early genetic experimental therapies is to measure the restoration of the defective protein in muscle, which involves a muscle biopsy, a significantly invasive procedure. It is therefore required to design and validate non-invasive markers, which allow measuring response to treatments in the ambulant and non-ambulant population.
Magnetic resonance imaging (MRI)has become a valuable tool to image the change in signal characteristics in dystrophic muscle. MRI provides non-invasive indices of muscle atrophy, fibro-fatty replacement, oedema, and structural abnormalities in DMD muscle that may offer valuable markers of efficacy for exon-skipping treatments. The time-course of these MRI indices are currently unknown, and particularly unclear in the non-ambulatory population.
The primary aim of this study is to 95uglieri9595ze through MRI differential involvement of muscle groups occurring with disease progression, more precisely by defining which muscle groups are best markers for therapeutic response in the non-ambulant boys. In addition we aim to define quantitative imaging changes in these muscle groups by using quantitative metrics for detecting change in these muscles over time (and, thus, by inference, with a therapeutic intervention) – ensuring that robust statistical methods are in place for interventional studies of non-ambulatory boys. A related aim of the study is to correlate the MRI findings with clinical assessments currently in use in the non-ambulant population.

This is a prospective longitudinal natural history study. Subjects with DMD would will be followed over a one year period. MRI datasets will be collected at baseline, 3 months, 6 months, and 12 months. Clinical observations will be performed at baseline, 6 months, and 12 months.
Healthy boys may also be scanned using MRI for the purposes of testing scan test/retest and for investigating differences in the MRI biomarkers between the DMD population and healthy boys. Healthy controls will be scanned either once or twice, for test/retest purposes.

All subjects will be asked not to undertake organised or unaccustomed physical exercise (i.e. participation in school sports, training clubs,) from 1 week before imaging and clinical assessments. This is to eliminate exercise as a confounding factor. Normal playing with friends is permitted.

For more information about the study please contact Dr Valeria Ricotti at v.ricotti@ucl.ac.uk.

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
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 understand 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@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

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)1 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@ucl.ac.uk.

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