Muscular Dystrophy Campaign

MRC Centre for Neuromuscular Diseases

UK Neuromuscular Translational Research Conference

25 -26 March 2010

Medical Sciences Teaching Centre
South Parks Road
Oxford OX1 3PL

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

UK Neuromuscular Translational Research Conference 2010

Dear Colleagues,

We are delighted to welcome you to Oxford for the third annual scientific meeting of the first MRC funded centre for translational research in neuromuscular diseases. We are very pleased that this annual UK Neuromuscular Translational Research Conference continues to be jointly hosted with the Muscular Dystrophy Campaign. This year we have also worked closely with the MRC Functional Genomics Unit in Oxford and with the Newcastle University Centre for Brain Ageing and Vitality (supported by the MRC and the BBSRC, EPSRC, ESRC) to devise what we hope is an innovative and interesting programme.

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

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

Over the next two days this conference aims to showcase a wide range of high quality scientific neuromuscular research from many UK groups, international colleagues and industry partners. There are focussed sessions on RNA therapies in neuromuscular diseases, on the application of MRI in neuromuscular diseases and on the latest developments in treatments for acquired autoimmune neuromuscular diseases. In addition, there is a collaborative session organised jointly by the MRC Centre and the Newcastle University Centre for Brain Ageing and Vitality addressing molecular and therapeutic aspects of age-related neuromuscular diseases and sarcopenia.

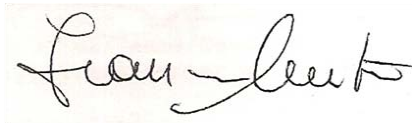
We have received over 90 high quality abstracts and there will be dedicated poster sessions each day as well as guided poster discussions. There will be four £500 poster prizes for young investigators. All accepted abstracts are published in the journal *Neuromuscular Disorders.*

Professor Angela Vincent will deliver the first John Newsom Davis lecture in recognition of John's enormous contribution to translational research in neuromuscular diseases. We are also delighted to welcome Professor Chris Kennard who will discuss translational research in the context of the MRC strategic plan.

We would like to thank the joint MRC-MDC meeting planning team, and especially Zoë Scott, 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.

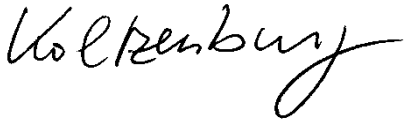
We sincerely hope that you have a stimulating and entertaining two days in Oxford.

Professor Michael G Hanna
Director
MRC Centre for Neuromuscular Diseases



Professor Francesco Muntoni
Deputy Director, ICH/GOS
MRC Centre for Neuromuscular Diseases

Professor Kate Bushby
Deputy Director, Newcastle
MRC Centre for Neuromuscular Diseases,



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

Professor Doug Turnbull
Director
Newcastle University Centre for Brain Ageing and Vitality

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

Welcome from Philip Butcher – CEO of the Muscular Dystrophy Campaign

Welcome to the 2010 UK Neuromuscular Translational Research Conference organised in partnership between the MRC Centre for Neuromuscular Diseases and the Muscular Dystrophy Campaign.

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

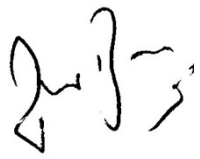
The Muscular Dystrophy Campaign has supported research into neuromuscular disorders for over 50 years. During this time our families and supporters have raised more than £50 million to fund cutting-edge science and research, whilst a further £50 million has been invested in care and support for families.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

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

This work is very much a team effort and the past 12 months has also seen 500 people living with muscle disease join forces with the Muscular Dystrophy Campaign to launch the National Muscle Group Support Network. The network consists of 12 individual 'Muscle Groups' which provide peer-to-peer support and secure new NHS investment and national media coverage. The groups are supported by over 100 MPs and 50 clinicians.

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

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



Mr Philip Butcher
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 list of current trials appears on page 60.

- 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 eight 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 all muscle disease. 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 muscle disease.

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

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, the focus of this research has begun to shift towards the search for treatments for these conditions.

Translational research involves a 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 the 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 will ultimately benefit the patient.

The Muscular Dystrophy Campaign aims to ease this transition by providing support to both scientists and clinicians. We fund basic research through to pre-clinical research and where possible clinical trials. As well as monetary help, we aim to provide a platform where clinicians and scientists can meet and discuss ideas.

One of our strategic aims is to fast-track promising treatment approaches when they are close to clinical trial and to ensure a rapid transition from bench to bedside. A major focus of the last year 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. We currently support research across a range of disciplines from the basic science through to research looking at treatment approaches for a number of conditions.

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 that these sessions will be of interest to you and 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

Day 1 – Thursday 25th March

09:00 – 10:15	**Registration**
10:15 – 10:30	**Introduction and Welcome** Professor Michael Hanna, MRC Centre for Neuromuscular Diseases UCL Institute of Neurology
10:30 – 12:30	**RNA Therapies in Neuromuscular Disease** Chairs: Professor Francesco Muntoni & Professor Kate Bushby
10:30 – 11:00	**An overview of RNA therapeutics** Professor GertJan B. Van Ommen, University of Leiden, The Netherlands
11:00 – 11:30	**RNA therapy approaches in myotonic dystrophy** Professor Charles Thornton, University of Rochester, USA
11:30 – 12:00	**Antisense approaches in SMA** Professor Ian Eperon, University of Leicester
12:00 – 12:30	**Results of a systematic antisense study in DMD** Professor Francesco Muntoni, UCL Institute of Child Health
12:30 – 14:00	**Lunch** UGa/b **Posters** Foyer, Classroom 1, UGc recess
14:00 – 15:30	**Focus on Acquired Neuromuscular Disease** Chairs: Dr David Hilton-Jones & Dr Michael Lunn
14:00 – 14:30	**Acquired inflammatory neuropathy** Dr Michael Lunn, National Hospital for Neurology and Neurosurgery
14:30 – 15:00	**Clinical and molecular aspects of dermatomyositis** Dr Lucy Wedderburn, UCL Institute of Child Health
15:00 – 15:30	**Clinical trials in myasthenia gravis** Dr Richard Barohn, Kansas University Medical Centre, USA
15:30 – 16:00	**The MRC and Translational Research:** **an update on the MRC's translational research strategy** Professor Christopher Kennard, Medical Research Council
16:00 – 17:15	**Tea** UGa/b **Posters** Foyer, Classroom 1, UGc recess
17:15 – 18:15	**The John Newsom-Davis Lecture introduced by Professor Dame Kay Davies:** **Acquired diseases of the neuromuscular junction** Professor Angela Vincent, University of Oxford
18:15 – 19:30	**Drinks reception and posters** Introduced by Philip Butcher, The Muscular Dystrophy Campaign
20:00 – 22:30	**Gala dinner , New College** Introduced by Professor Dame Kay Davies

Day 2 –Friday 26th March

08:45 – 11:15	**Joint MRC Centre Initiative: Age-related Neuromuscular Disease** Chairs: Professor Doug Turnbull and Professor Michael Hanna
08:45 – 09:00	**Importance of age-related sarcopenia – a joint centre initiative** Professor Doug Turnbull, MRC Centre for Neuromuscular Diseases, University of Newcastle
09:00 – 09:30	**Molecular changes in ageing muscle with relevance to neuromuscular disease** Professor Malcolm Jackson, University of Liverpool
09:35 – 10:05	**Inclusion body myositis – an age-related degenerative myopathy?** Dr Anthony Amato, Harvard Medical School, USA
10:10 – 10:40	**Protein aggregate myopathies and ageing** Professor Hanns Lochmüller, MRC Centre for Neuromuscular Diseases, University of Newcastle
10:45 – 11:15	**Motor neuron degeneration and its relationship to ageing** Professor Pamela Shaw, University of Sheffield
11:15 – 11:45	**Coffee** UGa/b
11:45 – 12:15	**New findings in FSHD** Chair: Prof Dame Kay Davies Dr Rabi Tawil, University of Rochester Medical School, USA
12:15 – 14:00	**Lunch** UGa/b **Posters** Foyer, Classroom 1, UGc recess
14:00 – 15:30	**New MRI Applications in Neuromuscular Disease** Chairs: Professor Tarek Yousry & Professor Volker Straub
14:00 – 14:30	**MRI in animal models of neuromuscular diseases** Professor Pierre Carlier, UPMC, Paris
14:30 – 15:00	**Paediatric applications of neuromuscular MRI** Dr Anna Pichiecchio, C. Mondino Institute of Neurology Foundation, Pavia
15:00 – 15:30	**Clinical applications of MRI in muscular dystrophy** Professor Volker Straub, MRC Centre for Neuromuscular Diseases, University of Newcastle
15:30 – 15:45	**Poster prize presentations & close**

Speaker Abstracts

An overview of RNA therapeutics

Gert-Jan B. van Ommen, Annemieke Aartsma-Rus, Maaike van Putten, Peter-Bram 'tHoen, Sjef Verbeek, Seda Yilmaz, Willeke van Roon, Melvin Evers, Jan Verschuuren§ Nathalie Goemans¶, Mar Tulinus#, Sjef de Kimpe*, Giles Campion* and Judith van Deutekom*. *Department of Human Genetics and (§)Department of Neurology, Leiden University Medical Center; Leiden NL, (¶)University Hospital Leuven, (#)University of Gothenburg SE, (*)Prosensa Therapeutics, Leiden NL*

Antisense-mediated reading frame restoration is presently the most promising therapeutic approach for Duchenne muscular dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing to restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional Becker-like dystrophin proteins. The approach is theoretically applicable to over 70% of all patients. We derived an algoritm to design skipping AONs aimed at intra-exonic sequences which outperforms other published approaches. Proof of concept has been achieved in cultured muscle cells from patients carrying different mutation types, in the *mdx* mouse and dog models and recently in patients as well. In a first trial published in 2007 (van Deutekom et al. NEJM) exon 51 skipping and dystrophin restoration was shown in four patients after local intramuscular AON injections. A subsequent systemic trial has recently been successfully completed by Prosensa, as a multicenter one-month dose-finding trial, using subcutaneous administration. This better route for self-administration was previously tested to be efficient in reaching proper muscle AON levels, with a lower peak circulation level as intravenous administration. The 12 patients enrolled in Belgium and Sweden showed a clear dose response, both of skipping and dystrophin restoration, without severe adverse effects. Our own preclinical research now focuses on the next steps in developing and improving therapy: the development of more refined readouts for therapeutic success using transcriptomics and proteomics technology in existing and tailored mouse models and supporting treatments to increase myogenesis. Finally, the success of the exon skipping work in DMD has led us to explore applications to genes affected in other diseases. Promising prelininary results have been obtained in several cases.

RNA therapy approaches in myotonic dystrophy

Charles Thornton, MD, *Department of Neurology, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Ave, Rochester, New York 14642, USA*

One of the most impressive features of skeletal muscle is its remarkable capacity for regeneration. In muscular dystrophy, however, this regenerative response is overwhelmed by ongoing muscle degeneration. Restoration of dystrophic muscle is theoretically feasible if the underlying molecular defect is corrected and regeneration is allowed to proceed. In practice this has not been accomplished because molecular defects in muscular dystrophy are difficult to correct and regeneration can be limited by fibrosis and inflammation. Myotonic dystrophy, however, may represent a special case. The initial histopathologic lesion is atrophy rather than necrosis, and there is relatively little fibrosis. Moreover, recent studies suggest that control of the disease process is feasible using RNA-targeted therapy. This presentation will highlight several approaches that have shown promise in preclinical testing, including antisense oligonucleotides that target a toxic transcript, small molecules that modulate RNA-protein interactions, and upregulation of RNA binding proteins.

Antisense approaches in SMA

H. Zhou[1*], N. Owen[2*], F. Muntoni[1] and I.C. Eperon[2] [1]*Institute of Child Health and Dubowitz Neuromuscular Centre, UCL* [2]*Department of Biochemistry, University of Leicester* *Equal contributors

Spinal muscular atrophy is an autosomal recessive disease, resulting from homozygous loss of function in the gene SMN1. A second gene, SMN2, can encode an identical protein, but a couple of single nucleotide differences from SMN1 prevent efficient splicing of exon 7, leading successively to a severe deficiency in the levels of functional protein, degeneration of motor neurons of the anterior horn in the spinal cord, weakness and atrophy of the proximal and axial voluntary muscles, limb and trunk paralysis and respiratory muscle weakness. Encouraging SMN2 exon 7 to splice more efficiently has been a major strategy for the development of a therapy.

Several approaches have been adopted. One relies upon the possibility that splicing is the outcome of complex combinatorial processes, i.e., that so many proteins assemble on exons and contribute in diverse and often opposing ways to the outcome that any given exon's splicing activity results from a unique combination of events. This suggests that small molecules might be found that stimulate splicing selectively of exon 7. A more direct approach to specificity relies upon base-pairing. Straightforward blocking of sequences that mediate negative regulation of splicing, to prevent the binding of cognate proteins, requires extensive experimental work to identify such sequences. Given that this is a constitutive exon in SMN1, it is surprising that many negatively-acting

sequences have been identified. We have developed a different strategy in which bifunctional oligonucleotides recruit activating proteins to the exon. We will describe recent results that demonstrate the potential of this method.

Results of a systemic antisense study in Duchenne muscular dystrophy

Muntoni F [1], Sebahattin Cirak S[1], Guglieri M[2], Arechavala V[1], Morgan J[1], Feng L[1], Torelli S[1], Bhardwaj N[1,] Sewry CA[1], Straub V[2], Shrewsbury S[3], Bushby K[2]. *[1]Institute of Child Health, London, UK; [2]Royal Victoria Infirmary, Newcastle upon Tyne, UK and [3]AVI BioPharma, Bothell, WA*

The UK MDEX Consortium (http://www.mdex.org.uk/) is involved in close collaboration with AVI BioPharma, in clinical trials using antisense oligonucleotides (AOs) to induce exon skipping in boys with DMD. In 2008 we completed a dose-escalation IM study of a morpholino (PMO) AO, AVI-4658, which induces skipping exon 51 in dystrophin mRNA. In February 2009 we initiated a dose escalation study in ambulant DMD boys aged 5-15 years with deletions benefitting from skipping exon 51. This study consists of 12 weekly administrations of AVI-4658 followed by a muscle biopsy to assess dystrophin expression at baseline and 14 weeks. Clinical parameters are followed for 26 weeks, consisting of safety (adverse events, physical examinations, laboratory tests), muscle, pulmonary and cardiac function, and pharmacokinetics at 1st, 6th and 12th doses. A Data Safety Monitoring Board guides dose escalation decisions. Cohorts 1-5 completed 12 weeks of dosing (January 2010), while cohort 6 will complete dosing in March 2010 and clinical observations in June. No drug related SAEs or severe drug related AEs have been reported so far. To date, single doses of 900mg and cumulative exposure exceeding 10,000mg have been well tolerated. Exon skipping and dystrophin protein expression in the cohorts analysed so far indicates a dose response in exon skipping and protein expression in the cohorts up to 4.0mg/kg, with data in the 10.0 and 20.0mg/kg cohorts available soon. These results suggest that AVI-4658 has the potential to lead to the development of a drug that could play a role in the treatment of DMD.

Acquired inflammatory neuropathy

Lunn, MP, *MRC Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK*

The inflammatory peripheral neuropathies are a heterogeneous group of conditions, some of which can be devastating and disabling. This lecture will update participants on the latest developments in common and less common inflammatory neuropathies encountered in practice.

Guillain-Barré syndrome, an acute monophasic post-infectious inflammatory polyradiculoneuropathy is the best understood in terms of pathogenesis. However the prognosis of this condition has not changed for 30 years with the mortality remaining at about 8-10%. New ideas for treatment based on increasing understanding of pathogenic mechanisms are emerging which may improve outcomes in the not too distant future.

Chronic inflammatory demyelinating polyradiculoneuropathy is a relapsing remitting or progressive condition which in most cases responds well to treatment. Intravenous immunoglobulin (IVIG) and steroids remain the backbone of treatment but the healthcare costs and long term side effect profiles are substantial. The ICE trial proved the effectiveness of IVIG in the long term. Future planned studies of rituximab and other long term immunosuppressants may provide curative treatment strategies.

Although no clinician will forget the patient with peripheral nerve vasculitis or POEMS syndrome, these remain 'orphaned' diseases amongst the inflammatory neuropathies. The evidence base for the treatment of peripheral nerve vasculitis is derived from renal and rheumatological medicine but provides good data on safety to support anecdotal regimens for treatment. The diagnosis of POEMS syndrome has become somewhat more straightforward with evidence that VEGF is dramatically raised in the serum and may mirror disease activity. Aggressive therapeutic management regimens culminating in peripheral blood stem cell transplantation appear to have good outcomes, in the short term at least.

Clinical and molecular aspects of dermatomyositis

Lucy R Wedderburn, *UCL Institute of Child Health, 30 Guildford Street, London, WC1N, UK*

Juvenile dermatomyositis (JDM) is the most common form of idiopathic inflammatory myopathy of childhood. Like adult DM, JDM affects skin and muscle but involves more serious complications such as calcinosis or ulcerative skin disease, than in adults. JDM may also cause serious morbidity through involvement of other organ systems including gut, lung and CNS. At present we do not have prognostic biomarkers with which to predict either response to treatment or development of serious complications. We have developed a system for standardised assessment of muscle biopsy tissue in JDM which is now being validated. Affected muscle tissue from children with JDM taken early in disease may look normal by standard histology but is already immunologically 'abnormal ' as evidenced by over expression of MHC Class l and deposition of C5-9 complex. We have demonstrated that juvenile

muscle is highly sensitive to MHC Class I protein over expression compared to adult muscle, using a transgenic model of over expression, and that this change induces ER stress. Data on the down stream effects of this insult are under investigation in model systems and patient tissue.

The John Newsom-Davis Lecture:
The neuromuscular junction - a wide spectrum of disease mechanisms
Angela Vincent, *University of Oxford, UK*

The neuromuscular junction (NMJ) is a relatively simple synapse between nerve and muscle that, being accessible to both circulating substances and to biopsy, has taught us many of the principles on which other synapses work. But in addition it exhibits a wide range of disease mechanisms - autoimmune, toxic and genetic.

John Newsom-Davis studied muscle biopsies initially to look at muscle spindle activity, following on from his work on respiratory physiology with Fred Plum in New York and Tom Sears in London. By happy coincidence, John was also directing the Batten intensive care unit at Queen Square where all the myasthenia gravis (MG) patients were taken after their thymectomies. A recommendation from Tom to Ricardo Miledi in the Biophysics department at UCL led to a collaboration between John and Ricardo that set the scene for all that followed. The autoimmune nature of MG was just being established in the USA, so John, with Tony Pinching and Keith Peters started plasma exchanging the MG patients (this leads to substantial reduction in circulating antibodies). There was a remarkable clinical effect which correlated inversely with the AChR antibody levels - that I had begun to measure - in almost every patient.

The exception was a young man who had had myasthenia since early childhood, and he had no AChR antibodies. John thought that he must have a genetic form of MG, and so we studied muscle biopsies from this and other patients, showing for the first time that congenital/inherited forms of myasthenia were distinct both clinically and, partly, electrophysiologically – leading to much excellent work in this condition by Andrew Engel, David Beeson, Hans Lochmüller and Daniel Hantai.

There were other MG patients, however, who did not have AChR antibodies but DID get better with plasma exchange. Many years later, we showed antibodies to muscle specific kinase (MuSK) in some of these. MuSK antibodies turn out to be interesting both clinically, as the patients have a rather striking phenotype, and functionally as we still don't understand really how the MuSK antibodies cause the NMJ defect.

John was always on the look out for new possible autoimmune disorders, and the Lambert Eaton myasthenic syndrome was a good candidate. He found that plasma exchange was effectively in these patients too, and Bethan Lang with Dennis Wray demonstrated that there were antibodies against the voltage-gated calcium channels on the presynaptic motor nerve terminals and that the associated small cell lung cancers expressed this antigen. The final autoimmune disease of the NMJ was not discovered until the 1990s when similar experiments, partly by Ian Hart, showed that acquired neuromyotonia was associated with antibodies to the presynaptic voltage-gated potassium channel. In all of these studies the importance of having specific neurotoxins that bind to the receptors and ion channels at the NMJ cannot be overstated. The lecture will try to give a historical perspective and update the audience on these conditions.

Reactive oxygen species and loss of muscle fibres during ageing
M. J. Jackson, T. Pearson, A. Vasilaki, G. Sakellariou, J. Palomero and A. McArdle, *School of Clinical Sciences, University of Liverpool, Liverpool, L693GA, UK*

Reactive oxygen species (ROS) appear to play a role in the fundamental processes underlying ageing and studies have examined the potential role of these species in the loss of muscle fibres that occurs during ageing. Skeletal muscle of young or adult animals has a remarkable capacity to adapt to an increase in the generation of ROS that occurs during physiological processes by up-regulation of a variety of regulatory proteins for ROS. During ageing these adaptive processes become ineffective and muscles of aged rodents shows no increase in the muscle content of antioxidant enzymes or heat shock proteins following contractile activity in comparison with that seen by muscle from adult or young rodents. In addition, ROS generation appears to increase with ageing since isolated mitochondria from muscle of ageing rodents show an increased release of hydrogen peroxide in comparison with mitochondria isolated from young rodents. Together these data suggest that restoration of the ability of muscle from aged organisms to respond to ROS might influence age-related loss of muscle mass and function. This possibility has been examined by genetic overexpression of heat shock proteins in muscle of mice. This manipulation has been found to prevent the age-related loss of muscle force production seen in aged mice indicating potential therapeutic approaches that may reduce loss of muscle mass and function in elderly human subjects.

Inclusion body myositis – a age related degenerative myopathy?

Anthony Amato, MD. *Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115, USA*

Inclusion body myositis (IBM) is the most common idiopathic inflammatory myopathy occurring in patients over age 50. Muscle biopsy characteristically reveals endomysial inflammation, small groups of atrophic fibers, eosinophilic cytoplasmic inclusions, and muscle fibers with one or more rimmed vacuoles. However, any given biopsy may lack these histopathological abnormalities so the key to diagnosis is the pattern of involvement of muscle groups on clinical examination. Early and often asymmetrical weakness and atrophy of the quadriceps and flexor forearm muscles (i.e., wrist and finger flexors) are the clinical hallmarks of IBM. The pathogenesis of IBM is unknown. It may be autoimmune inflammatory myopathy or a primary degenerative myopathy with a secondary inflammatory. Some investigators purport that proteins that accumulate in brains of patients with Alzheimer disease are evident by immunohistochemstry in IBM muscle. This has led to the theory that β-APP is overproduced in IBM muscle fibers, that this is somehow cleaved into abnormal β-amyloid, and the accumulation of the later or "tau" is somehow toxic to muscle fibers. However, there are problems with this theory. Unfortunately, IBM is generally refractory to therapy. Further research into the pathogenesis, along with both preliminary small pilot trials and larger double-blind, placebo-controlled efficacy trials are needed to make progress in our understanding and therapeutic approach for this disorder.

Protein aggregate myopathies and ageing

Hanns Lochmüller, *Institute of Human Genetics, Newcastle University, International Centre for Life Newcastle upon Tyne, NE1 3BZ, UK*

While many forms of inherited muscle disorders primarily affect children, hereditary inclusion body myopathies (hIBM) and myofibrillar myopathies (MFMs) are almost exclusively seen in adults. Depending on the exact genetic defect, first symptoms start between the 3rd and the 7th decade, suggesting a role of ageing in the pathomechanism. The 2 most frequently found forms of inherited IBM in the UK are caused by mutations in GNE and in VCP. GNE mutations are associated with the recessive form of hIBM, characterized clinically by foot drop and sparing of the quadriceps muscle. VCP mutations cause an autosomal dominant form of IBM that is often associated with Paget's Disease and with FrontoTemporal Dementia (IBMPFD). Myofibrillar myopathies (MFM) are characterized by specific morphological changes and caused by mutations in several genes (such as the desmin and the myotilin-encoding genes), most of them Z-disc related. In addition, MFM frequently present rimmed vacuols. Protein aggregates are frequently observed in these disorders reminiscent of protein aggregates in age-related disorders of the brain.

New Findings in FSHD

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The genetic lesion in >95% of patients with FSHD, a loss of an integral number of subtelomeric D4Z4 repeats (<11 repeats) on chromosome 4qter, was first described in 1993. Yet, until recently, the underlying molecular mechanism remained an enigma. The D4Z4 repeats, contains an open reading frame, *DUX4*, for which, it has been difficult to demonstrate a stable transcript or protein in somatic cells. An alternative hypothesis was that loss of a critical number of repeats, a buffer between heterochromatic and euchromatic DNA, led to dysregulation of genes centromeric to the repeats. However, no consistent, reproducible change in centromeric gene expression was found. Several lines of evidence now point back to *DUX4*. The *DUX4* gene is evolutionarily conserved and several *DUX4* transcripts and proteins are produced by the D4Z4 repeat. In addition, pathogenic contraction of the D4Z4 repeats occurs on a distinct 4qter haplotype (4qA161) and is associated with a permissive change in chromatin structure limited to the repeat array. Supporting a pathogenic role for the chromatin changes is the occurrence of D4Z4-restricted hypomethylation in a subpopulation of FSHD (FSHD2), in whom no contraction in the number of D4Z4 repeats occurs. The current prevailing hypothesis for the molecular mechanism of FSHD postulates that the permissive chromatin changes at D4Z4, whether or not associated with loss of a critical number of repeats, results in perturbation in the basal expression of *DUX4*. The *DUX4* a pro-apototic protein, can interfere with normal myogenesis and make cells more susceptible to oxidative stress.

NMR imaging and spectroscopy in animal models of neuromuscular diseases

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In patients with neuro-muscular disorders, NMR imaging is useful to 1/ assist diagnosis by identifying topographical patterns of muscle involvement and 2/ determine muscular lesion progression in longitudinal studies. NMR

investigations of animal models focus on this second objective and aim at characterizing precisely muscle degenerative and/or inflammatory changes during disease progression and at assessing muscle responses to treatment. Translational research benefits from the NMR indices of pathological features defined and validated in experimental models, and secondarily transferred to studies in humans. Another possibility with the NMR techniques is to take advantage of the non-invasive characterization of muscle in order to optimize therapeutic deliveries and select processes with minimal toxicity.

Muscle trophicity and fatty degenerative changes can be precisely measured using 3D sequences, such as 3pt Dixon or selective excitation GE, which separate muscle tissue from fatty infiltration. Fibrosis can be determined using advanced quantification schemes, which compensate for signal distortions introduced by instrumental imperfections. Other approaches, from T1-rho or ultra-short TE sequences to Gd-molecules with high affinity for collagen, aim at a more direct visualisation of the fibrotic component. A quantitative evaluation of muscle inflammation/oedema can be achieved using T2 mapping. Sarcoplasmic membrane leakiness in models of dystrophinopathies can be assessed by time-course studies of muscle T1w signal enhancement after injection of Gd-chelates, possibly coupled to albumin. Phosphorus NMR spectroscopy might provide more specific indices of membrane integrity, which need to be validated versus histology and tested in pre-clinical therapeutic trials.

Paediatric applications of neuromuscular MRI

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In the last decade there has been increasing evidence of the role of muscle MRI in identifying disease specific patterns. We will review our experience in several neuromomuscular disorders. We will also report the results of a recent study reporting excellent sensitivity and specificity of muscle MRI in identifying patterns of muscle impairment. . Our findings suggest that muscle magnetic resonance imaging could be used in clinical practice as an additional tool in the differential diagnosis of muscle disorders with prominent spinal rigidity.

Clinical applications of MRI in muscular dystrophy

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Because of the non-invasive nature of MRI and its excellent properties to detect muscle pathology at high resolution, it has become a useful tool in the diagnostic workup of patients with muscular dystrophy. MRI can help to guide muscle biopsies and to assess the extent and degree of muscle pathology. MRI is also complementing the clinical examination by delineating disease specific patterns of muscle involvement in the muscular dystrophies. In recent years muscle MRI has been applied to also quantify muscle pathology, which makes it an interesting tool to provide outcome measures for monitoring treatment effects. There are still several challenges though before MRI can be used as a reliable outcome measure in longitudinal multi-centre studies. There is currently a lack of standardized operating procedures to measure quantitative changes and there is no consensus how to perform data analysis.

T1w-images are presently the clinical gold standard to qualitatively assess muscle involvement on a 6-point scale and can be rapidly acquired on all MRI systems. Assessment of T_2 is often desired as a measure of structural change, particularly oedema. In muscular dystrophies with fat infiltration, however, fat suppression is required to provide additional information to T1w scans and this is presently difficult to achieve in a uniform way that can be implemented in a multi-centre setting. Alternatively the 3 point-Dixon technique for quantitative fat measurements can be used to obtain quantitative maps of muscle fat content on a scale of 0-100% fat content for each muscle and allows easy comparison (i) between centres in a multi-centre study and (ii) maximum statistical power in a longitudinal treatment study. The technique minimises the effect of B0 and B1 inhomogeneities present in the standard T1w images allowing accurate quantification.

We will present data from tow MRI studies performed in muscular dystrophy patients. In a study to quantify the differences between normal and corticosteroid-treated Duchenne muscular dystrophy (DMD) lower limb muscle we used signal intensity measurements on T1-weighted and gadolinium contrast-enhanced images. To investigate the effect of moderate exercise we also measured T2-values. We will also present preliminary data from a multi-centre study on limb girdle muscular dystrophy 2I (LGMD2I), in which we used the 3 point-Dixon technique to quantify fat infiltration and phosphorous MRS to compare and contrast inorganic phosphate (Pi) and phosphodiesters (PDE) between muscles that showed involvement in the disease (gastrocnemius and soleus) and those that were spared (tibialis anterior).

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Poster Abstracts

Muscular Dystrophies: DMD – Molecular Therapy

Poster 1

Current progress with the systemic administration trial of AVI-4658, a novel Phosphorodiamidate Morpholino Oligomer (PMO) skipping dystrophin exon 51 in Duchenne muscular dystrophy (DMD)

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Objective: AVI BioPharma in collaboration with the MDEX consortium have identified a PMO to skip dystrophin RNA exon 51 in DMD patients, restore the reading frame and enable expression of dystrophin protein. Here, we test 6 PMO doses to select an effective, well tolerated dose for subsequent registration.

Method: Open label, dose escalation study in ambulant DMD boys aged 5-15 years with relevant deletions, of 12 weekly administrations of AVI-4658; 14 week follow up with muscle biopsy to assess dystrophin expression. Clinical efficacy (including 6 minute walk and North Star assessment), skeletal muscle, pulmonary and cardiac function is being assessed. Safety assessment includes adverse events, physical examinations and laboratory tests – including hematology, coagulation studies, chemistry and anti-dystrophin antibodies. A DSMB guided dose escalation decisions (across 6 doses: 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg).

Results: Study fully enrolled 19 patients by Dec 2009. All doses well tolerated (ongoing at 20mg/kg). No Drug Related SAEs or severe AEs reported so far. To date, maximum single dose is 900mg and cumulative PMO dose exceeds 8100mg. Biopsies from first 4 cohorts showed exon 51 skipping at 2 and 4 mg/kg and 1 patient with 20% increase in number of dystrophin positive fibres.

Conclusion: Study drug well tolerated to date. Dosing and follow up continue on schedule. These preliminary data bode well for safe long-term administration of AVI4658 in DMD boys, and suggests clinically meaningful dystrophin expression can be expected following systemic administration. Preliminary, laboratory data from the remaining cohorts are due in 2Q 2010.

Poster 2

Multiexon skipping in Duchenne muscular dystrophy

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Duchenne musclular 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, 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. However, the different deletions that cause DMD would require skipping of different exons, and personalised molecular medicine may be required. As DMD deletions appear to be concentrated in the region around exons 45 and 55 (65% of all DMD mutations), multiexon skipping has been proposed as a means to treat the maximum number of patients with one formulation of AOs. We describe here studies in cultured human skeletal muscle cells to optimise the skipping of exon 45-55 block, using linked AOs tagged with hnRNP A1 binding sites, and polypyrimidine tract binding protein binding sites. This work will be extended in vitro in cultured DMD patient cells and in the humanised DMD mouse, a transgenic mouse that expresses full length human dystrophin.

Poster 3

The characterisation of out of frame duplications in DMD patients

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Introduction: Duchenne muscular dystrophy (DMD) is an X-linked recessive disease caused by mutations in the dystrophin gene, leading to disruption of the reading frame. Out-of-frame deletions are the most common mutations (65%) but out-of-frame duplications are also frequent (at least 15% of all DMD mutations). Although there is no treatment at the moment, restoring the reading frame using antisense oligonucleotides (AOs) has been shown to

be effective in early “exon skipping” trials in DMD boys with out of frame deletions. However, the application of AOs to the duplicated patients has its own unique problems. Firstly, these AOs not only recognise duplicated exons specifically, but also recognise normal exons. Also, the skipping could be problematic in patients with big duplications.

This study focuses on the characterization of splicing patterns in patients with duplications (inverted or non-inverted duplication, tandem duplication), which will help to evaluate the feasibility of exon skipping strategies to restore the disrupted reading frame in these patients.

Method: We used frozen sections, fibroblast or myoblast cell lines from 16 patients with proven duplications. We used several combinations of primers targeting the areas adjacent to the duplication and then the presence of the right products was confirmed by sequencing. Nested PCR was necessary for the patients with more then two duplicated exons.

Results: All the patients in this study showed non-inverted duplications and tandem.

Conclusion: Traditional PCR method has been used to characterize the duplications in 16 patients and this is the first step to design the AOs that will be tested as a potential therapy for the DMD patients.

Poster 4

Lentivirus-mediated stem cell therapy for Duchenne muscular dystrophy

Jacqueline Jonuschies, Luisa Boldrin, Francesco Muntoni and Jennifer Morgan *The Dubowitz Neuromuscular Centre, Institute of Child Health, University College London, UK*

Duchenne Muscular Dystrophy is a genetic disorder characterized by loss of dystrophin leading to progressive muscle fibre degeneration, finally resulting in failed muscle regeneration. One possible therapeutic approach is to deliver viral vectors that can transduce skeletal muscle and replace dystrophin. Satellite cells (SCs), stem cells residing underneath the basal lamina of myofibres, play a central role in skeletal muscle regeneration. Long-term therapeutic benefit could only be achieved by restoring dystrophin protein expression not only in muscle fibres but also in SCs, thereby maintaining healthy muscle fibres throughout life. Lentiviruses hold great potential as a gene therapy tool for skeletal muscle, as they can stably integrate their genomes into dividing and non-dividing cells, and provide long-term expression. However, low transduction efficiencies in muscle and early promoter silencing *in vivo* have been discouraging. Here, we show that primary SC cultures can be transduced with lentiviral vectors requiring moderate MOIs. Lentiviral transduction does not affect SC myogenicity, and transgene expression is maintained for at least 3 weeks in culture. Interestingly, transduction of single myofibres *in vitro* revealed GFP expression in both associated SCs and the myofibre syncytium. Next, we will compare muscle-specific (e.g. Desmin) and silencing-resistant (e.g. 2AUCOE) promoters with our results of a strong viral promoter, and determine the level and longevity of transgene expression in SCs and myofibres *in vitro* and *in vivo*. Finally, we will investigate the potential of transduced SCs engrafted into immunodeficient mice to repair and regenerate skeletal muscle *in vivo*.

Poster 5

Induction of dystrophin in Duchenne muscular dystrophy patients by antisense oligonucleottide AVI-4658 restores the dystrophin-associated glycoprotein complex

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We have recently performed a proof of principle single-blind, controlled, two-doses escalation study of a morpholino splice-switching oligonucleotide (AVI-4658) which induced skipping exon51 in dystrophin mRNA in seven patients with DMD[1]. The morpholino was injected into one extensor digitorum brevis (EDB) muscle while the contralateral muscle received saline. No adverse effects resulted from injection of AVI-4658; in all patients exon 51 skipping was demonstrated at the RNA level, and the higher-dose of AVI-4658 resulted in increased dystrophin protein expression in all treated muscles. Although the intensity of dystrophin immunolabelling was not uniform, it increased up to 42% of that of healthy muscle.

We now report the expression of proteins from the dystrophin-associated glycoprotein complex (DGC) in the dystrophin-positive fibres

Methods: The DGC proteins were studied by immunofluorescence with antibodies to dystrophin (dys1, dys2, dys3 and Mandys106),α-sarcoglycan, β-dystroglycan, nNOS, and dystrobrevin, and compared with β-spectrin, utrophin and neonatal myosin and other markers of immaturity such as laminin α5. Further assessment of the effect of regeneration, quantification by immunoblotting and quantification of immunofluorescence is in progress.

Results The increased detection of dystrophin in the treated muscle was accompanied by more intense immunofluorescent labelling of the DGC. Intensities were variable and differences due to different affinities of the antibodies were taken into account.

Conclusion . We show that dystrophin expression induced by AVI-4658 is followed by restoration of the DGC in these fibres, further indicating that the shortened dystrophin produced in these patients is functional.

1. Kinali M, et al. Lancet Neurol. 2009;8:918-928

Muscular Dystrophies: DMD – Molecular Therapy

Poster 6

Evaluation of the truncated products of exon and multiple exon skipping in DMD therapy

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Duchenne's muscular dystrophy (DMD) is a severe muscle wasting disorder affecting 1/3500 male births. DMD is caused by mutations in the DMD gene leading to a lack of dystrophin protein in skeletal muscle resulting in a breakdown of the integrity of the muscle cell membrane. The resultant muscle fibres are highly prone to contraction induced injury. Consequently the progressive rounds of degeneration and regeneration of the muscle lead to the replacement of muscle fibres with non contractile fibrotic tissue and fatty infiltrates. These alterations lead to progressive muscle wasting, weakness and death in late adolescence. Gene therapy strategies for the delivery of dystrophin to skeletal muscle have been hampered by a number of factors.

A promising alternative therapeutic approach for DMD is antisense-mediated exon skipping using antisense oligonucleotides (AONs) targeting specific exons to restore the DMD reading frame. The products of these therapies are truncated forms of dystrophin, which should restore the integrity of the muscle cell membrane and elevate the degeneration of muscle fibres. An ideal therapy could target multiple exons, thereby treating many more patients whilst still producing a partially functional truncated dystrophin protein product. Some of these AONs are currently in clinical trial for single exon skipping.

In order to evaluate the therapeutic value of these therapies, several different forms of truncated human dystrophin were cloned into the pCI plasmid. These truncated forms represent the dystrophins created by skipping different single exons or skipping multiple exons currently being investigated by various labs. The truncated dystrophins were electro-transferred into *mdx* mice muscle and their expression was assessed. Truncated dystrophin resulting from skipping exon 45 to exon 55 is expressed in mice muscle and correctly localises to the sarcolemma.

Poster 7

Translation related clinical trials in duchenne muscular dystrophy (DMD) in the UK

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A number of studies have moved to the bedside in the form of phase l/ll/III clinical trials in DMD.

ANTISENSE OLIGONUCLEOTIDE (AO) AVI-4658 The MDEX Consortium in collaboration with AVI BioPharma is conducting AO trials in London and Newcastle. A phase l/lb proof of concept IM study in 7 DMD boys, funded by DoH is complete (Kinali et al 2009). A phase l/ll systemic, open-label, dose escalating safety study in 19 DMD boys in six cohorts and jointly funded by MRC UK and AVI-BioPharma is ongoing. Preliminary data from this study will be presented at the meeting.

ATALUREN (PTC124) This is a phase 2b efficacy and safety study in DMD with Nonsense-Mutations. It is an international, multicenter, randomized, double-blind, placebo-controlled study. It recruited 174 subjects from 37

centres, including 21 in Newcastle, Oswestry and London. This study is complete and preliminary analyses indicate no significant difference in primary endpoint between treatment and placebo groups. Analyses of muscle dystrophin expression are ongoing.

DMD HEART PROTECTION This is a double-blind randomised multi-centre, placebo-controlled trial of combined ACE-inhibitor and beta-blocker therapy in preventing the development of cardiomyopathy in DMD without echo-detectable left ventricular dysfunction. It is funded by BHF with centres in Newcastle, London, Oxford, Oswestry and Birmingham. The study will recruit 140 patients for a five year treatment period. Primary outcome will be measured through echocardiography.

These and other planned clinical studies mark the beginning of a new era for clinical trials in DMD which will hopefully open the way forward for improved treatment and survival of affected boys.

Poster 8

Exploring emotional impact in a proof-of-principle single-blind, controlled, two-doses escalation intramuscular study of a morpholino splice-switching oligonucleotide (AVI-4658) trial to induce dystrophin restoration in children with Duchenne muscular dystrophy

Elena M. Garralda[1], Maria Kinali[1], Sebahattin Cirak[2], Francesco Muntoni[2] *[1]Imperial College London, UK [2]The Dubowitz Neuromuscular Centre, UCL Institute of Child Health London, UK*

Objective: Previous researchers have noted depressive reactions in neuromuscular patients taking part in proof of concept trials. We developed a tool to assess risk of adverse emotional reactions and tested it in eight children with DMD who participated in our recently completed proof-of-concept study[1].

Methods: The emotional risk tool had 11 items and quantified 1) family expectations, psychosocial stress, function, emotional reactivity, psychiatric history; 2)current child psychiatric status. Assessment included interviews/questionnaires with parents and children at trial entry and completion (when emotional impact as assessed).

Results: The mean child age was 12.9 years (6 were using wheelchairs). Mean total risk score was 3.13 (2.3). One family with the highest risk score (7/11) revealed unreasonable expectations from the trial and withdrew participation. Five families returned follow-up impact questionnaires. On an 11-point Lykert scale (10 highest impact) the mean child emotional impact score was 2.0 (2.3): the two children with the highest scores (4 & 7/10) reported an increase in anxiety symptoms but their parents did not note deterioration in psychiatric status. The mean reported impact on mothers was 1.9 (2.4). Child and parent impact was significantly associated with total risk scores (especially so with psychosocial family stress and to a lesser extent with family communication difficulty at study entry).

Conclusion: Emotional impact from the trial was reported for a minority of children and was predicted by our risk tool. Impact scores were associated with only limited change in child psychiatric adjustment. Reference: 1. Kinali M, et al. Lancet Neurol. 2009;8:918-928

Muscular Dystrophies: DMD – Clinical Management/Networks

Poster 9

A Novel Ankle foot orthoses/ footwear combination to aid walking in Duchenne Muscular Dystrophy

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This pilot study will investigate the use of a novel AFO/footwear combination that supports dynamic equinus in ten boys with Duchenne Muscular Dystrophy (DMD). To maintain independent walking, boys with DMD use equinus gait for optimal alignment of the ground reaction force through the hip and knee joints. As the disease progresses, there is reduced ability to compensate for muscle weakness. This new orthotic strategy is based on understanding DMD gait and the impact of ankle foot orthoses on lower limb biomechanics during walking. It has been trialled with good effect in one boy with DMD. This project for ten boys with DMD will examine user opinion on the novel ankle foot orthosis, quantify its effect on walking parameters using three dimensional movement analysis and measure changes in activity levels.

Poster 10

Parental Stress Levels in Parents of Children with Muscular Dystrophy

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It has been suggested that children and adults with conditions, including Duchenne muscular dystrophy, myotonic dystrophy and congenital myotonic dystrophy, would particularly benefit from the input of a clinical psychologist to help families develop effective management strategies (Yoshimura, Sasaki, Akimoto & Yoshimura (1989). In Shropshire a clinical psychologist was integrated into the Neuromuscular Service in September 2008 and the effectiveness of psychological intervention is being assessed through prospective audit. As there is currently no baseline information on stress levels in parents of children with muscular dystrophy that use the service, a research project which aims to assess this is currently being developed. It hopes to establish whether there are differences in the levels of stress experienced by parents whose children have been diagnosed with a specific neuromuscular condition, for example Duchenne muscular dystrophy or congenital myotonic dystrophy; and also whether gender differences exist between parents. We intend to use a measure of 'Childhood Illness-Related Parenting Stress' the Paediatric Inventory for Parents (PIP) (Streisand, Braniecki, , Tercyak & Kazak, 2001) which measures levels of stress in parents of children with a critical illness. It consists of 42 questions based upon difficult events which parents of children who have (or have had) a serious illness sometimes face. Using the 5 point scale (1=Never, 2=Rarely, 3=Sometimes, 4=Often, 5=Very often), parents are asked to indicate a) how often the event occurred in the past 7 days, and b) how difficult it was/or generally is for them to cope with (1=Not at all, 2=A little, 3=Somewhat, 4=Very much, 5=Extremely). Data will be analysed with a One-way analysis of variance (ANOVA) and the results will allow us to determine whether there are significant differences for (a) condition and (b) gender. The findings will help guide the delivery of care provision and service development.

Poster 11

UK NorthStar Neuromuscular Clinical Network (NSCN): National Audit results in Duchenne Muscular Dystrophy (DMD) Corticosteroid practice, Vitamin D status and bone health

Adnan Y Manzur, Elaine Scott*, Pinki Munot, Kayal Vijaykumar, Francesco Muntoni, on behalf of UK NorthStar Clinical Network. *Dubowitz Neuromuscular Centre, Department of Paediatric Neurology, Great Ormond Street Hospital for Children & Institute of Child Health, MRC Muscle Centre, London. *Muscular Dystrophy Campaign, UK.*

The North Star Clinical Network (NSCN) is a collaboration of seventeen UK centres. The network aim is to develop consensus on best DMD clinical management, with agreed assessment and treatment protocols. Longitudinal data regarding DMD is collected in a web-based database, which enabled these national audits:

Corticosteroid treatment in DMD in UK: Data from 240 ambulant boys with DMD (age range 3-18 years) is available. Median age at diagnosis was 4.1 years. 223 were treated with corticosteroids; median age at steroid initiation was 6.3 years (N=203). Starting corticosteroid was prednisolone in 203 and Deflazacort in 10. Starting corticosteroid regime was intermittent (10 days on, 10/20 days off) in 117 and daily in 83 patients.

Vit D status prior to corticosteroid treatment: Vitamin D levels in 157 boys. 25 OH Vit D levels were deficient (> 37.5 nmol/L) in 91 boys (58%) and insufficient (37.5 – 50.0 nmol/L) in another 31 boys (20%).

Vertebral Fractures (VF) in corticosteroid treated DMD: VF occurred in 30 steroid treated boys at a mean age of 11.5 years (range 7.1-15.5); 78% were symptomatic. Mean latency, from start of steroids to VF, was 4.1 yrs (0.7-7.4). 28/30 were on daily corticosteroid regime at the time of VF. These results informed 2009 ENMC workshop recommendations for bone health in DMD.

The NSCN and its database provide a unique tool to optimise clinical practice on a national level and facilitate translational research.

Acknowledgements: The support of Muscular Dystrophy Campaign for the NSCN is gratefully acknowledged. The full list of NSCN is available at: http://www.muscular-dystrophy.org/research/news/1259.

Poster 12

An audit of bone density and vertebral fractures during steroid treatment in Duchenne Muscular Dystrophy

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Osteoporosis and an increased fracture risk are common in Duchenne Muscular Dystrophy(DMD). Corticosteroid therapy(CS) given to prolong mobility increases the severity of osteoporosis and risk of vertebral fractures which can have serious functional consequences.

We reviewed case notes of 22 patients with DMD, to examine the effect of CS on BMD(spinal BMD z-scores) and vertebral fractures and oral residronate treatment .

Results: 19/22 patients were treated with corticosteroids. Mean age at start of treatment was 7.93 yr(5.03-15.39) and mean steroid duration was 4.1 years(0.5-6). During the steroid treatment eight fractures (two vertebral and six long bones) were reported. Two patients had vertebral fractures (one asymptomatic and one traumatic) reported on routine X-ray screening. A greater number of patients demonstrated minor vertebral changes. Mean time interval between starting steroids and vertebral fractures was 4.47 yr(4.07-4.87).

Mean spine BMD z-score was not low before or within 3 months of start of steroid treatment, mean -1.12(2.12 to -3.13). At 2 years post steroid treatment BMD z-score was significantly lower, mean -1.6(0.7 to -3.3; binomial test, $p<0.01$)

On the basis of early X-ray changes and BMD seven patients on steroids were treated with 35 mg oral risedronate, fortnightly. Of the two vertebral fractures reported, one occurred while the patient was on residronate. All patients tolerated oral residronate without side effects.

Conclusions: Boys with DMD, treated with steroids, are at risk of fractures. In steroid-treated boys, BMD z-score was significantly low after 2yr. The incidence of vertebral fractures in this study group was low (10% vertebral fracture rate for 77.9 patient-years of steroid treatment). This may be due to early intervention with residronate, based on BMD and X-ray screening. Fortnightly oral residronate was well tolerated.

Muscular Dystrophies: Limb Girdle Muscular Dystrophy

Poster 13

Identification of a novel group of muscular dystrophies, the Anoctaminopathies, caused by recessive mutations in the putative calcium activated chloride channel, ANO5.

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The Anoctamin (ANO) family consists of 10 proteins several of which have been shown to correspond to the elusive calcium-activated chloride channels (CaCCs). CaCCs are gated by increases in intracellular calcium and they have been linked to several cellular functions including epithelial transport, cell volume regulation, olfactory and photoreceptor transduction, cardiac membrane excitability, and smooth muscle contraction. The only reported human mutations linked with the ANO family are dominant mutations in *ANO5,* which cause a rare bone fragility disorder gnathodiaphyseal dysplasia (GDD1). Recently we have identified recessive *ANO5* mutations in patients with proximal limb girdle muscular dystrophy (LGMD2L) and a distal non-dysferlin Miyoshi myopathy (MMD3). The mutations identified consist of splice site, a single adenine duplication and missense. The duplicated adenine is present in LGMD2L and MMD3. The LGMD2L phenotype is characterized by proximal muscle weakness and prominent asymmetric quadriceps atrophy. The MMD3 phenotype is associated with distal weakness in particular of the calf muscles. The clinical heterogeneity associated with ANO5 mutations is reminiscent of that observed with dysferlin mutations which can cause both a LGMD and distal muscular dystrophy.
ANO5 mutations are associated with loss of muscle membrane integrity and defective membrane repair. Our studies suggest that ANO5 is a putative calcium-activated chloride channel which may function with dysferlin in membrane repair. Our study has identified a novel group of muscular dystrophies "the Anoctaminopathies".

Poster 14

The First UK Family with *Ano5*-Associated Myopathy

Anna Sarkozy[1*], Gareth Marlow[2*], Rumaisa Bashir[2], Debbie Hicks[1], Rita Barresi[3], Steve Laval[1], Hanns Lochmuller[1], Volker Straub[1] and Kate Bushby[1] * These authors contributed equally *1) Institute of Human Genetics,*

University of Newcastle, Newcastle upon Tyne, U.K.; 2) School of Biological and Biomedical Sciences, University of Durham, Durham, UK; 3) NCG Diagnostic & Advisory Service for Rare Neuromuscular Diseases, Muscle Immunoanalysis Unit, Dental Hospital, Newcastle upon Tyne, UK

Limb girdle muscular dystrophy type 2B and Miyoshi myopathy (MM) are allelic and overlapping autosomal recessive conditions, the majority of which are caused by dysferlin gene mutations. Mutations in the *ANO5* gene have recently been identified in non-dysferlin MM families. *ANO5* encodes a member of the Anoctamin family of proteins, many of which have been shown to be calcium-activated chloride channels. The Anoctamin family all share 8 transmembrane domains, which lead to their earlier classification as the Transmembrane Protein 16 (TMEM16) family. Here we describe a UK family with *ANO5*-associated myopathy. The index patient, a 54 year old female, showed first symptoms at age 29 years, with difficulties standing on toes and pain in heels. The condition slowly progressed and in her mid 30's the patient showed inability to toe walk, difficulty walking upstairs and reduced exercise tolerance. At age 47 years the patient showed distal lower limb wasting, proximal and distal lower limbs muscle weakness, in particular in plantar dorsiflexion. Her sibling started showing difficulties getting up on her tiptoes and reduced shoulder power from her mid 40's. At age 51 years, she showed mild distal lower limbs weakness but no muscle wasting. CK values of the two patients were elevated at 1800 and 4000 IU/l, respectively. Muscle biopsy of the index patient showed mild fibre size variation and internal nuclei in some fibres. Dysferlin immunostaining was normal. The parents were unaffected and unrelated. Both siblings carry a homozygous variant in exon 5, c.191dupA. It is predicted that this variant would lead to a frameshift and premature truncation, with the mutant protein lacking all transmembrane domains. This variant segregates with disease in the family, providing further evidence that this is likely to be the disease causing mutation.

Poster 15

Modelling the Role of Dystroglycan Glycosylation in Angiogenesis using zebrafish

Alasdair Wood, Catherine Jepson, Steve Laval, Kate Bushby, Hanns Lochmüller, Rita Barresi, Juliane Müller, and Volker Straub *Institute of Human Genetics, Newcastle University, UK*

Mutations in the genes encoding the putative glycosyltransferases fukutin and fukutin-related protein (FKRP) cause various forms of muscular dystrophy. These muscle diseases have variable severity, but are all associated with defective glycosylation of α-dystroglycan. The "vascular hypothesis" of muscular dystrophy (MD) predicts that the muscle necrosis associated with MD is caused by chronic muscle ischaemia. Dystroglycan is known to be essential in vascular development and so deficiency in FKRP and fukutin was investigated using transgenic zebrafish expressing EGFP in the blood vessel endothelium (fli-1) by treatment with translation-blocking oligonucleotide morpholinos. At 1 day post fertilisation (dpf) the eye vasculature in both morphants was distorted, correlating with the severity of the phenotype. In the embryos with the most severe phenotypes at 3 dpf the eye areas were significantly smaller than controls. In the mildly affected morphant embryos the somitic intersegmental vessels failed to reach the dorsal longitudinal anastomosis. With increasing severity of phenotype the intersegmental vessels retracted further and in some cases were found to be missing. Fukutin knock downs had a more severe effect on the vascular phenotype. Downregulation of FKRP and Fukutin in TG*(fli1:EGFP)* zebrafish may provide a good model system to investigate how pathologies associated with dystroglycan glycosylation relate to angiogenesis.

Poster 16

National Commissioning Group for rare neuromuscular disorders: LGMD diagnostic and advisory service in Newcastle

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Many muscular dystrophies that appear very similar by clinical assessment are known to be caused by defects in different genes. Typical examples are the limb-girdle muscular dystrophies (LGMDs), progressive diseases of muscle that produce weakness in a limb-girdle distribution. The LGMDs are now known to be caused by at least seven genes with dominant inheritance (LGMD1A-G) and fifteen genes that are inherited in a recessive manner (LGMD2A-O). Newcastle leads the National Commissioning Group (NCG) Diagnostic and Advisory Service for rare neuromuscular disorders, and is specifically responsible for providing Limb-Girdle Muscular Dystrophy (LGMD) diagnostics and advice.

The service includes:

* Diagnosis, assessment and treatment of patients with known or suspected rare neuromuscular disorders;
* Detailed clinical assessments;
* Specialised neurophysiological tests;
* Immunological analyses on tissue biopsies;

* DNA analysis of specific genes associated with the disorders and some overlapping clinical phenotypes.

The definitive diagnosis for a patient is ultimately resolved by identifying the primary gene defect. At the moment it is not possible to undertake DNA analysis alone for the majority of LGMDs without prior protein analysis, because the number of possible genes for study is so large. Also other forms of muscular dystrophy with a similar phenotype to LGMD have to be excluded. Ongoing involvement with research is essential, since relevant disease-causing genes are still being discovered.

Muscular Dystrophies: FSHD

Poster 17

Investigating the molecular mechanisms of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an inherited muscular disorder estimated to affect about 7/100,000 people. Two phenotypically identical but genetically distinct subtypes are recognized. FSHD1 patients have a contraction of the 4q35 macrosatellite repeat array D4Z4 (1-10 units rather than the usual 11-150), while FSHD2 is caused by an as yet unidentified mutation. In both subtypes, the disease is associated with altered chromatin structure of D4Z4 that is proposed to perturb expression of genes near to or within the array. The *DUX4* open reading frame, which is located within each individual D4Z4 repeat unit, encodes a putative protein containing two conserved homeodomains and is presumed to act as a transcription factor. Our research focuses on investigating D4Z4 sequence variations as well as analysis of DUX4 transcripts and protein products.

We aim to map potential *in vivo* binding sites by Chromatin-Immunoprecipitation combined with next-generation sequencing (ChIP-Seq). Currently, DUX4 has no confirmed in vivo targets, although there is *in vitro* evidence for binding in the promoter region of the PITX1 gene. In addition to confirming PITX1 as a DUX4 target, sequencing of V5-ChIP enriched DNA should identify additional candidate genes for further functional studies.

Our group has also identified a D4Z4 homologous locus in the mouse and comparative ChIP experiments on the mouse Dux homologue will allow us draw conclusions on human DUX4 functions and may shed light on its role in the molecular disease mechanism of FSHD.

Muscular Dystrophies: Myotonic Dystrophy

Poster 18

Variant triplet repeats in the CTG expansion of DMPK affect stability of the expanded region and may contribute to unusual symptoms observed in some myotonic dystrophy type 1 cases

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Myotonic dystrophy type 1 (DM1) is the most common type of muscular dystrophy and is caused by the expansion of a highly unstable triplet repeat (CTG) in the 3' untranslated region in the DMPK gene. DM1 symptoms are highly variable in both severity and the range of tissue-specific symptoms presented. Previously we have determined that an unusual Dutch family co-segregating DM1, Charcot-Marie-Tooth disease, encephalopathic attacks and early hearing loss, carries a complex expansion containing variant CCG and GGC repeats at the 3' end of the expansion. The variant repeats at the 3' end remain relatively stable while the 5' CTG region remains genetically unstable and prone to expansion. We have now identified complex variant repeats in the 3' end of the CTG expansion in a

number of unrelated DM1 cases that also present with unusual symptoms. We suggest that the interruption of the CTG expansion by such variable repeats contributes both to the unusual symptomatic variation and severity of disease observed in some unusual cases of DM1.

Poster 19

An extremely high rate of *de novo* base substitution mutations causes interruptions at the myotonic dystrophy type 1 locus

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Recently us, and others reported novel GC rich arrays interrupting the canonical runs of CTG triplets at the DM1 locus in a subset of patients with unusual molecular diagnostic results. In the present study we tested the prevalence of these variant repeats by screening 142 DM1 patients with apparently typical symptoms and molecular test results. We observed three different interruptions within or adjacent to the CTG triplet in seven patients (4.9%). These were a G:C > C:G transversion in the immediate 3' flanking sequence, CGG and CCG triplets. To investigate the origins of these alleles, we traced their transmission across generations in these patient's families. Here we show, for the first time, that these interruptions originated as *de novo* base substitution mutations in three paternal germlines at the rate of between 1.4×10^{-4} and 4.5×10^{-4} mutations/base/generation. This rate is 1000 to 100,000 fold higher than background in the human genome, and 10 fold higher that at the loci that cause achondroplasia and Apert syndrome, two well characterized base substitution hotspots. Therefore, in addition to being a hotspot for length mutations, the DM1 locus is also a base substitution hotspot. An analysis of the genotype-phenotype correlation showed that the interruptions conferred varying levels of stability to the DM1 allele, and appeared to affect disease onset in one family. This is in line with previous findings. As interruptions could have an effect on the genotype-phenotype correlation, they should be accounted for in DM1 diagnostic tests and research.

Poster 20

Screening for drugs to treat Myotonic dystrophy

David Brook, Chris Hayes, Thelma Robinson, Inyang Udosen, Sukrat Arya and Javier Granados Riveron *Institute of Genetics, University of Nottingham*

Myotonic Dystrophy (DM) is the most common form of muscular dystrophy in adults, affecting around 1 in 5,000 people. It is a progressive neuromuscular disorder in which patients suffer from muscle weakness and wasting, and a variety of other symptoms including cardiac arrhythmias, diabetes, cataracts and frontal balding in males.

The molecular events underlying myotonic dystrophy have been characterized and the disease mechanism can be broken down into a series of stages that provide possible points for therapeutic intervention. The primary molecular event in DM occurs at the DNA level. DM1 is caused by a CTG repeat expansion and DM2 by a CCTG repeat expansion. In both cases the repeat sequences are greatly expanded in patients' cells and the extent of expansion generally correlates with disease severity. The next stage of the disease is at the RNA level. The expression of DMPK in DM1 and ZNF9 in DM2 produces transcripts containing CUG and CCUG repeat expansions, respectively, which remain in the nucleus of DM cells forming distinct foci. We have developed an assay using *in situ* hybridization with fluorescently labelled single stranded oligonucleotide probes complementary to the repeat expansion transcripts to identify the nuclear foci.

We have established a series of fibroblast cell lines from more than 20 DM patients. Four of these have been stably transfected with a telomerase-expressing plasmid to allow continued growth in culture and two of the lines have also been infected with an inducible plasmid to express MyoD, which promotes differentiation into myoblasts and myotubes. We have used these cells in a medium through-put assay using a tissue culture liquid handling robot that allows the analysis of up to 4,000 wells per week. In situ hybridization images are analysed on a Molecular Devices high content imaging system and data are scored using an algorithm for focus number, area and intensity. Three different chemical libraries are currently being screened.

Muscular Dystrophies: Other

Poster 21

The Relationship between Syntrophins and Syntrophin-Binding Sites (SBSs) in the Dystrophins and Dystrobrevins

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Duchenne muscular dystrophy (DMD) is a lethal multisystem disorder that results from defects in dystrophin and the consequent disruption of the dystrophin glycoprotein complex (DGC). Although the basic function of the DGC remains unclear, attempts have been made to explain many aspects of the DMD phenotype in terms of the loss of proteins normally recruited to the DGC by the syntrophin proteins. Syntrophins are adaptors which bind to multiple modular sites (SBSs) in the core DGC proteins dystrophin, utrophin, DRP2 and α- and β-dystrobrevin. Syntrophins have also been implicated in long QT syndrome and multiple sclerosis.

Alternative splicing is used to modulate the number of SBSs in dystrophin, and we have recently shown it can also modulate not only the number but also the type of SBSs in α-dystrobrevin. This raises the possibility that there is significant specificity of interaction between syntrophins and SBSs, and therefore that both the stoichiometry and "flavour" of DGC syntrophins can be modulated.

The human genome encodes five syntrophins and eleven SBSs. We here describe the preliminary characterisation (using quantitative interaction studies, mutagenesis and comparative biology) of the extent and determinants of their specificity of interaction, and discuss the implications for DGC function in various tissues.

Poster 22
Clinical and pathological heterogeneity in partial merosin deficiency
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Background: Recessive mutations in the *LAMA2* gene leading to complete laminin α2 deficiency underlie a severe phenotype of congenital muscular dystrophy type IA (MDC1A), a disorder characterised by muscle weakness and white matter changes on brain MRI. We describe a family with a syndrome characterized by slowly progressive myopathy, cortical white matter changes and epilepsy.

Case report: The proband presented with a history of epilepsy and progressive proximal muscle weakness. Her one affected brother exhibited a slowly progressive myopathy but not epilepsy. An MRI scan of her brain revealed diffuse white matter changes consistent with leukodystrophy. Histology of her affected muscle from both affected individuals demonstrated a reduction in laminin α2 staining. In addition, there were features suggestive of inclusion body myopathy including rimmed vacuoles and inclusion bodies. Genetic studies of the *LAMA2* gene identified two new pathogenic heterozygous point mutations (c.2749+1G>A; p.Cys393Gly) in both individuals. Her two unaffected siblings were carriers for the splice site change but not the missense mutation.

Conclusions: The presence of compound heterozygous mutations in *LAMA2* together with a reduction in laminin α2 staining confirms the diagnosis of partial merosin deficiency in the two affected individuals. The presence of epilepsy in the proband and its absence in the affected brother suggests that other genes are also likely to determine the phenotype. In addition, the finding of features typical of inclusion body myopathy on muscle histology in both affected patients suggests that merosin deficient muscular dystrophy is associated with a wide spectrum of clinical and pathological features.

Mouse Models of Neuromuscular Diseases

Poster 23
Assessing the effects of exercise-induced stress on the Fiona mouse model
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Background and Aim: Characterized by the severe progressive wastage of skeletal muscle, Duchenne muscular dystrophy is a crippling disease that is caused by the absence of the cytoskeletal protein dystrophin. *Utrophin* is a paralogue of *dystrophin*. The Fiona mouse is an *mdx* (dystrophin-deficient) transgenic mouse that over-expresses the full-length utrophin protein in skeletal muscle. Various studies have shown that it is completely rescued and does not display any of the dystrophic characteristics of *mdx* mice. However, these studies have only been performed on sedentary mice. Our aim was to see if Fiona mice continue to display this rescued phenotype after an extended period of sustained exercise-induced stress, or whether they revert to the dystrophic phenotype.

Methods: 4-week-old C57BL/6, *mdx*, and Fiona mice were divided into two groups – 'sedentary' and 'run'. Those in the 'run' group were made to run on a treadmill at12 $m.min^{-1}$ for 30 minutes, twice a week, for 8 weeks. After the end of the trial, muscle samples were dissected out and subjected to a range of tests.

Results: Muscle physiology tests show a significant decrease in maximum isometric force produced by the extensor digitorum longus (EDL) muscle caused by exercise in *mdx* and Fiona but not C57BL/6 mice. Leftward shifts in the force-frequency curves were seen for all groups. Increased centronucleation was seen in muscle sections of *mdx* mice but not of C57BL/6 and Fiona. These data indicate that utrophin's protective effect is partially diminished after a sustained period of exercise-induced stress.

Poster 24

Blocking calcium influx with streptomycin worsens myocardial pathology in the *mdx* mouse model of muscular dystrophy

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There is evidence to suggest that abnormal calcium influx is involved in the pathology of Duchenne muscular dystrophy (DMD) and short term treatment with the nonselective calcium channel blocker has been shown to ameliorate muscle pathology in the *mdx* mouse model of the disease. We have investigated whether early (in utero) and long term (until 6 months of age) treatment with streptomycin can prevent/ameliorate muscle and heart pathology in mdx mice. We present histological and functional MRI data to suggest that long term treatment with streptomycin does not improve heart pathology. On the contrary, treated animals showed evidence of increased myocardial sarcolemmal damage, necrosis and fibrosis. Treated mice showed no evidence of improved left ventricular function with overall trends toward reduced cardiac function. Treated C57/BL10 mice had significantly reduced left ventricular mass and cardiac output suggesting that the treatment had negative effects on healthy controls that showed no histological evidence of myocardial damage. The potential for non-selective calcium channel blockers as a therapy for DMD is therefore questionable.

Poster 25

Utrophin Luciferase Knock-in Mouse Model for *in vivo* Assessment of Drug Efficacy in Preclinical Trials for Utrophin Upregulation

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder caused by mutations in the cytoskeletal protein dystrophin. By pharmacologically upregulating the dystrophin-related protein utrophin, our aim is to develop a therapy for DMD by reconstructing the dystrophin-associated protein complex. BMN-195 (SMT C1100) - the lead compound identified from our recent small compound screening programme - has recently entered Phase I trials in humans. Preclinical screening for utrophin upregulation in the *mdx* mouse is complicated by large variations in background levels of utrophin. In order to circumvent this problem and expedite the preclinical screening process we have generated a new mouse model in which a luciferase reporter has been knocked into one utrophin allele. The reporter is under the control of the endogenous utrophin regulatory region including both promoters A and B. The other allele remains intact to provide all the therapeutic utrophin necessary to compensate for the lack of dystrophin. Quantification of the level of luminescence being emitted from this model *in vivo* during a drug trial will enable assessment of drug efficacy without having to sacrifice the animal and terminate the trial. Variation in endogenous utrophin between littermates can be assessed prior to trial enrolment. The ability to prolong the length of promising trials and prematurely terminate those where efficacy is low should dramatically improve the throughput of *in vivo* preclinical drug trials for utrophin up-regulation in the *mdx* mouse.

Poster 26

Rescue of Severely Affected Dystrophin/Utrophin deficient mice by Morpholino-Oligomer mediated exon skipping

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Duchenne Muscular Dystrophy (DMD) is a severe neuromuscular disorder caused by mutations in the dystrophin gene that result in the absence of functional protein. Antisense-mediated exon skipping is one of the most promising approaches for the treatment of DMD because of its capacity to correct the reading frame and restore dystrophin expression which has been demonstrated *in vitro* and *in vivo*. In particular, peptide-conjugated

morpholino oligomers (PPMO) have recently been shown to induce widespread high-levels of dystrophin expression in the *mdx* mouse model.

In this study, we have investigated for the first time the therapeutic potential of PPMO in the utrophin/dystrophin double-knockout mouse (dKO) which is a much more severe and progressive mouse model of DMD. Repeated intraperitoneal injections of a PPMO targeted to exon 23 of dystrophin pre-mRNA in dKO mice induce a near-normal level of dystrophin expression in all muscles examined, except for the cardiac muscle, resulting in a considerable improvement of their muscle function and dystrophic pathology. PPMO treatment strikingly prevented kyphosis and contractures in dKO mice and remarkably improved their motility. Treated dKO mice showed almost return to normalcy for most of the examined parameters as well as an extended lifespan, suggesting great potential for PPMO in systemic treatment of the DMD phenotype.

Poster 27

Chronic long term administration of phosphorodiamidate morpholino oligomer profoundly ameliorates activity, muscle strength and phenotype in dystrophic mdx mice

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DMD is characterized by premature termination of dystrophin translation and the administration of properly designed antisense oligonucleotides (AOs) can restore the correct reading frame in the dystrophin transcript. This approach in humans can potentially convert the DMD to the milder Becker Muscular Dystrophy phenotype. Due to the nature of this approach, chronic administration of AOs for all the life of the patient would be necessary. The phosphorodiamidate morpholino oligomer (PMO) is one of the most promising AO chemistries thanks to the high affinity to the sequence target and the resistance to endonucleases which reduce the number of administrations and allow a long lasting exon skipping.

In this study mdx mice were systemically treated with 2 different dosages distributed in 20 injections in a time of 12 months: a low dose which represents a clinically applicable amount of PMO and a high dose to verify the eventuality of toxic effects.

PMO was systemically injected in 6 weeks old animals. Mice were sacrificed 4 and 12 months after the beginning of the treatment. Skeletal muscles showed widespread dystrophin expression and significant histological improvement. Creatine kinase assay, in situ force measurement of muscle strength and open-field behavioural activity monitoring test showed a substantial amelioration of the dystrophic phenotype. Biochemical assays demonstrated no toxic effects after long term PMO administration.

Our results support the clinical feasibility of this approach with naked PMO.

Poster 28

Muscular dystrophy begins early in embryonic development deriving from stem cell loss and disrupted skeletal muscle formation

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By examining embryonic myogenesis in two functionally related skeletal muscle dystrophy mutants (*mdx* and *cav-3*$^{-/-}$) we establish that the pathology of Duchenne muscular dystrophy (DMD) and limb-girdle muscular dystrophy type 1C (LGMD-1c) originates in the disruption of the embryonic cardiac and skeletal muscle patterning processes. Myogenesis is severely disrupted and occurs earlier in *mdx* (DMD model) than in *cav-3*$^{-/-}$ (LGMD-1c model) and includes developmental delay; myotube morphology and displacement defects; and aberrant stem cell behaviour. These data are consistent with the milder phenotype of LGMD-1c, and the earlier (E9.5) embryonic expression of dystrophin. Stem cell defects (hyperproliferation and apoptosis of Myf5+ and attrition of Pax7+ myoblasts) occur in both *cav-3*$^{-/-}$ and *mdx*, from E15.5 and E11.5, respectively, both mutants have cardiac defects. Several *mdx* embryo pathologies have reciprocity with *cav-3*$^{-/-}$ mutants and caveolin-3 protein is elevated in *mdx* embryos. In double mutant (*mdxcav-3*$^{+/-}$) embryos where caveolin-3 is reduced below WT levels, phenotypes are severely exacerbated: intercostal muscle fibre density is reduced by 71%, and Pax7-positive cells are depleted entirely from the lower limbs and severely attenuated elsewhere. These data establish a key role for dystrophin in early muscle formation and demonstrate that caveolin-3 and dystrophin are essential for correct fibre-type specification and emergent stem cell function. These data plug a significant gap in the natural history of muscular dystrophy and will

be invaluable in establishing an earlier diagnosis for DMD/LGMD and in designing earlier treatment protocols, leading to better clinical outcome for these patients.

Poster 29

Preventing dystroglycan phosphorylation as a route to therapy in DMD

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Dystroglycan is a central component of the dystrophin glycoprotein complex (DGC) of striated muscle, mediating essential connections between the extracellular matrix and the actin cytoskeleton of muscles. No muscle disease involving mutations in dystroglycan itself have so far been described, however mutations in genes that post-translationally modify dystroglycan give rise to a class of diseases known as the dystroglycanopathies. All dystroglycanopathies currently characterised arise from proteins involved in the glycosylation of a-dystroglycan, a post-translational modification that is essential for dystroglycan function in binding to laminin in the ECM. However b-dystroglycan is also post-translationally modified, by glycosylation and by phosphorylation. *In vitro* analyses have demonstrated that phosphorylation of b-dystroglycan on tyrosine targets b-dystroglycan for degradation and this could be part of the mechanism that underlies loss of dystroglycan and the whole DGC in conditions such as DMD. Restoration of dystroglycan in DMD could prevent loss of DGC components and allow other compensatory proteins such as utrophin and plectin to bind to and stabilise the complex, thus ameliorating the DMD phenotype. We are using dystroglycan-null zebrafish and a new knock-in dystroglycan mutant in mouse to test the restorative effect of preventing dystroglycan phosphorylation on a key regulatory tyrosine. The homozygote dystroglycan knock-in mice are phenotypically normal with no obvious signs of muscle pathology. The knock-in has been crossed with *mdx* and analyses are in progress to determine any affect on pathology.

Poster 30

The integrin effectors talin 1 and 2 are essential for skeletal muscle development and integrity

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Integrins are essential for development and maintenance of skeletal muscle. Mutations in α7-integrin cause congenital myopathy in humans, and integrin ablation causes muscular dystrophy and defects in myofibre development in mice. Talin 1 and talin 2 mediate a connection between integrins, actin and signaling proteins, and in muscle, they concentrate at the myotendinous junction (MTJ). We have used genetically modified mice to identify the specific functions of the talin genes in muscle development. Ablation of either talin 1 or talin 2 leads to a myopathy characterized by the detachment of myofilaments from the sarcolemma at the MTJ. Defects are more pronounced in talin 2-null mice, which present with centrally nucleated fibers, and appear not to be caused by an increase in sarcolemmal damage as observed for example in *mdx* mice. Interestingly, the phenotype of talin2-null mice resembles that of α7-integrin-null mice, which also present centrally nucleated fibres with only a moderate increase in serum creatine kinase. Ablation of both talin isoforms causes severe developmental defects, with impaired myoblast fusion and myofibrillogenesis. Together, the data reveal an essential function for talin1 and talin 2 in muscle development and integrity. The similarity of the phenotype of talin 2- and α7-integrin-null mice suggests that mutations in talin 2 could lead to a congenital myopathy similar to that caused by α7-integrin mutations, and suggests that this gene should be considered as a candidate in patients without an identified genetic defect.

Poster 31

Myofibrillar myopathy caused by a mutation in the mouse *Myh4* gene

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Ariel is a mouse mutant selected from a cohort of ENU mutagenized mice owing to the onset of hind-limb paralysis at post-natal day 12 in homozygotes. Histopathology showed the presence of large protein aggregates and myofibrillar degeneration in skeletal muscle. The mutation, identified by positional cloning, causes a L349G change within the motor domain of MYH4 (MyHC IIb), the most abundant skeletal muscle myosin in the adult. Biochemical analysis of the aggregates indicated the presence of proteins found to be mutated in myofibrillar myopathies (FilaminC, ZASP and A/B-Crystallin) and ultrastructural analysis showed predominantly disorganized filamentous material. Transfections *in vitro* using GFP tagged versions of the proteins, showed that over-expression of MYH4(L349G) in a non-sarcomeric cell line produced twice as many cells with intracellular aggregates compared to MYH4, suggesting that the pathogenic mechanism involves a change in the folding of the motor domain. In fully differentiated myotubes, overexpressed MYH4 is incorporated into the A-band effectively, but MYH4349G forms aggregates. Intriguingly, the purely recessive nature of the mutation indicates that dimers of wild type and mutant

MYH4 assemble into thick filaments and that this is sufficient to prevent the onset of molecular pathogenesis. This mouse model represents a useful resource to elucidate the mechanisms of myofibrillar degeneration and to study the prevention of protein aggregates formation.

Poster 32
Investigating novel mutant mouse models of motor neuron disease
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Mutations in Tar DNA binding protein (TDP-43) have been identified as causes of both sporadic and familial motor neuron disease. TDP-43 is a ubiquitous, multi-domain and multifunctional nuclear protein and is crucially involved in gene expression, development and RNA metabolism. It remains unclear as to why mutations in the protein selectively cause motor neuron death. However since it has been identified as a cause of motor neuron disease, interest has heightened as to whether aberrant RNA metabolism could lead to selective motor neurone degeneration.

We have access to two lines of mutant TDP-43 mice (K160R, Q101STOP), produced by ethylnitrosourea (ENU) mutagenesis. We will initially investigate these mice with a combination of *in vitro* and *in vivo* techniques. Firstly, embryonic motor neuron cultures will be prepared and stress granule formation will be assessed. Stress granules are discrete cytoplasmic granules, containing untranslated mRNAs, dynamically formed following cellular insult as an anti-apoptotic mechanism. We will determine whether the TDP-43 mutations affect stress granule formation and cell viability. Secondly, muscle strength and number of functional motor units of the Tibialis Anterior and Extensor Digitalis Longum will be assessed in anaesthetised adult animals to determine whether the TDP-43 mutations cause motor neuron degeneration and muscle wasting.

Poster 33
Analysis of small fibre function in the C3 mouse, a model of CMT1A
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CMT1A neuropathy caused by duplication of the PMP22 gene is generally thought to affect large myelinated fibres. It is however reported that elevated thermal thresholds signalled by thin myelinated and unmyelinated fibres are commonly found in these patients. We therefore analysed the properties of sensory neurons in the C3 mouse, a novel mouse model of CMT1A with 3 or 4 copies of PMP22. Using the skin-saphenous nerve-preparation *in vitro* we found a reduction in the conduction velocity and amplitude of A-fibres. To further investigate the properties of distal A- and C-fibre terminals we used immunohistochemistry to study intra-epidermal nerve fibre density and Merkel cell distribution and their innervations by myelinated fibres. We found a significant reduction in intra-epidermal nerve fibre density indicating that there is a distal loss of unmyelinated fibres. Futhermore, there is a trend towards a reduced number of Merkel cells in the glabrous skin indicative of Abeta fibre innervations. In addition, we investigated whether the functional properties of the cell body of sensory neurons were affected. DRG neurons from all spinal levels were dissociated and kept in short term culture. There was no change in cell size distribution indicating that there is not a selective loss of sensory neurons. However, there was a significant reduction of neurons stained with isolectin B4, a marker for non-peptidergic unmyelinated fibres and a significant increase in cells binding the Cholera toxin B subunit. These changes in unmyelinated fibres qualitatively resemble those after axotomy. Using Fura ratiometric calcium imaging we studied the functional properties of neurons to TRP channel agonists which are selectively expressed in unmyelinated and thin myelinated fibres. There was no significant change in the percentage of neurons responding to the TRPM8 agonist menthol, or the TRPA1 ligand mustard oil. However there was a significant reduction in the percentage of neurons responding to the TRPV1 agonist capsaicin expressed on heat sensitive neurons.

Poster 34
Exploring IGFN1 in Cardiac Muscle
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IGFN1 was identified as an interacting partner of the sarcomeric protein, KY, the loss of which underlies a form of postural muscle-specific muscular dystrophy in the mouse (*kyphoscoliotic* mutant). IGFN1 has a predicted domain composition comparable to that of other sarcomeric support proteins including filamin C, myosin binding protein C and titin. Disruption within these genes is associated with muscular dystrophies and cardiomyopathies hence,

IGFN1 may also contribute to the degenerative molecular pathways common to these striated muscle-specific diseases.

In this work, northern analysis revealed that *Igfn1* is exclusively expressed in skeletal muscle, with smaller isoforms expressed in both skeletal muscle and heart. Several proteins have been identified as putative cardiac IGFN1 products with molecular weights of 189kDa, 135kDa and 64kDa, the 135kDa isoform being the most predominantly expressed form. Antibodies recognising C-terminal epitopes of IGFN1 localise to the intercalated disk in heart and primary cultured murine cardiomyocytes. Ultrastructural analysis refined this localisation to the adherens junction. Furthermore, peptide mass fingerprint analysis and liquid chromatography followed by tandem mass spectroscopy of IGFN1 immunoprecipitated complexes identified N-cadherin, beta-catenin and plakoglobin, three well-characterised proteins of the adherens junction, as interacting partners of the 135kDa IGFN1 variant. The molecular roles of IGFN1 remain to be established hence a conditional targeting vector has been generated and used to generate chimeras; matings to confirm germline transmission of the targeting sequence are currently underway. Once available, this knockout will help clarify the role of IGFN1 at the intercalated disk.
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Muscle Satellite Cells

Poster 35
Immortalisation and Characterisation of Muscle Stem Cells
Morten Ritso, Louise Jørgensen, Steve Laval, Volker Straub, Kate Bushby and Hanns Lochmüller *Institute of Human Genetics, Newcastle University, UK*

SJL-*Dysf* and mdx mice are well-established models for investigating dysferlin-deficient and dystrophin-deficient muscular dystrophies, respectively. *In vivo* mouse studies are important research methods, yet time-consuming and costly. Establishing primary myoblast cell lines from these disease-model and control mice – here C57BL/10 – will provide a valuable tool to investigate cellular and molecular paths and mechanisms involved in muscular dystrophies. Potential treatments can also be tested, allowing efficient analysis and screening strategies without the use of animals.

Myoblast cell lines were derived from C57BL/10 control, mdx and C57BL/10.SJL-*Dysf* mice. These mice had been crossed into the background of the “Immorto-mouse”, which express a genetic construct constituting a thermolabile large T antigen controlled by the IFNγ-inducible H2K^b promoter. Hence, permissive growth conditions were used throughout with IFNγ in growth medium and a set temperature of 33°. Myoblast isolation was achieved successfully using an adaptation of a standard enzymatic digestion protocol. The subsequent derivation of single-cell clones was optimised to large area plating rather than employing the conventional limiting dilution method.

The obtained cell colonies were induced to differentiate for testing the ability to fuse and form myotubes. Additionally the cells were analysed with immunofluorescence for expression of Desmin and Myogenin, both well-known myoblast markers. The presence of these markers in several populations of C57BL/10-Immorto control as well as mdx-Immorto and C57BL/10.SJL-*Dysf*-Immorto confirmed their myogenic nature. Despite the expression of orthodox markers determining these clones as myoblasts, it will still have to be demonstrated that these cell lines will be proliferating after multiple passages and will not become senescent.

Poster 36
Phenotypic separation of satellite stem cells using flow cytometry
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Satellite cells (SCs) are the principal muscle stem cells. They are defined by their anatomical location under the basal lamina of myofibres (Mauro, 1961) and are physiologically quiescent. Upon activation SCs proliferate to form a pool of muscle precursor cells, express myogenic regulatory factors and differentiate to repair or replace damaged or lost muscle fibres (Zammit et al, 2006). It is known that SCs are a heterogeneous population on the basis of marker expression, such as CD34 and M-cadherin (Beauchamp et al, 2000). Furthermore, only a small proportion of satellite cells regenerate skeletal muscle (Collins et al, 2005) and these are likely to be satellite stem cells. We have been fractionating either freshly isolated or cultured SCs by flow cytometric sorting on the basis of different phenotypes, including proliferative state, expression of low or high reactive oxygen species and different cell surface markers. Here we report limitations and advantages of the separation criteria used so far. In vitro characterization of the different sorted SC sub-populations is extending our knowledge about SC biology and stem cell state. Finding a way to enrich the stem cell fraction of SCs would be a step forward for cell therapy in muscle diseases.

Poster 37

Pax3/Pax7 transcriptional activity is *in vivo* required for muscle differentiation

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Satellite cells, the principal muscle stem cells, are characterized by expression of two crucial paired-box factors, Pax3 and Pax7, required during the developmental and adult myogenesis. Constitutive expression of Pax3 or Pax7 either in satellite cells or in C2C12 myoblasts lacking either protein, causes increased cell proliferation and decreased cell size. Accordingly, expression of dominant-negative constructs leads to reduced cell division, combined with a dramatic increase in cell size. Interestingly, following grafting into irradiated muscles in mdx nu/nu host mice, C2C12 cells that had been retrovirally infected with Pax3/Pax7 constructs are able to contribute to muscle regeneration, but, if dominant-negative constructs are constitutively expressed, the myogenic cells do not differentiate into myofibres.

These findings show that Pax3/Pax7 transcriptional activity is *in vivo* required for myogenic differentiation, and provide evidence for an important role in their coordination of cell growth and division.

Poster 38

Mice lacking lamin A/C have disorganised myotendinous junctions and perturbed satellite cell function

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Mutations in *LMNA*, encoding the nuclear lamina proteins lamin A/C, underlie several muscle diseases, including autosomal Emery-Dreifuss muscular dystrophy (A-EDMD). While most *LMNA* mutations are missense and act in a dominant manner, the absence of functional lamin A/C also causes a severe myopathy. Here, we have examined muscle structure and satellite cell function in a mouse model of A-EDMD, the *lmna* null mouse.

We found widespread morphological abnormalities in myonuclei, with variability in size, shape and chromatin distribution. The morphology of the myotendinous junctions was disturbed with less inter-digitation between myofibre and tendon, together with disorganised sarcomeres and collagen/lipid deposits. Muscle contraction parameters in *lmna*$^{-/-}$ mice were also dramatically impaired. These abnormalities may contribute to the early contractures that occur in A-EDMD, prior to muscle weakness.

Lamin A/C is expressed in satellite cells: the resident stem cells of skeletal muscle. Satellite cells from *lmna* null mice exhibited abnormal morphology and chromatin distribution. Analyzing activation, proliferation and differentiation, revealed a delay in satellite cell activation in *lmna*$^{-/-}$ mice. Satellite cell-derived myoblasts from these mice could differentiate efficiently, but nuclear morphology was abnormal even in these newly formed myotubes. Therefore, perturbed satellite cell function may contribute to both development and disease progression in A-EDMD.

Poster 39

Satellite Cell heterogeneity

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Our knowledge of satellite cell biology originates largely from embryonic and young animal models. It is assumed that throughout adult life and across populations, these myogenic stem cells, located between the basal lamina and the sarcolemma of muscle fibres, regenerate muscle by recapitulating generic developmental processes.

However, it has become increasingly apparent that satellite cells are a heterogeneous population both at the inter cellular and the inter individual level. Our studies aim to investigate recently described satellite cell markers, including VCam-1, Calcitonin, Notch 3 and M-cadherin, to better characterise heterogeneity within the satellite cell niche. Based on these findings we will then quantify the distribution of satellite cells, the expression of novel and typical markers and the regenerative capacity of satellite cells from different mouse populations. We are particularly interested in comparing dystrophic and wild type, male and female, and young and aged muscle. This will be achieved by immunostaining freshly-isolated myofibres, satellite cells cultured either on their fibre, or removed from their fibre and cryosections of mouse muscles. Quantification of satellite cell heterogeneity and the

observation of how this changes with age may help to elucidate therapeutically beneficial subpopulations and provide valuable insight into age related mechanisms of muscle regeneration.

Poster 40

Dystrophin expression in DMD pericytes after infection with U7 lentivirus designed to skip dystrophin exon 51

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Transplantation of autologous genetically modified stem cells has been proposed as a treatment for dystrophies such as Duchenne Muscular Disease (DMD). Pericytes, stem cells associated with blood vessels, have been shown to be a promising muscle stem cell. Whether such cells, derived from DMD patients with deletions in particular dystrophin exons, could be transfected with constructs designed to skip the appropriate exon has not yet been reported. Here, we have generated a highly myogenic pericyte cell line (pD2) from skeletal muscle of a DMD patient with a deletion of dystrophin exons 45-50, and infected the cells with different MOIs of lentivirus containing an U7 construct designed to skip exon 51. Our data showed that lentiviral infection had no detrimental effect on the differentiation of pericytes into myotubes *in vitro*. Infected cells skipped exon 51 as shown by nest RT-PCR and differentiated myotubes derived from these cells contained dystrophin protein. Our data provided evidence that human pericytes derived from DMD muscle could be genetically modified to express dystrophin *in vitro* without interfering with their myogenic capacity, thus providing a promising cell source for transplantation. The contribution of these cells to muscle fibres that express dystrophin is being tested in an immunodeficient mouse model.

Poster 41

Analysis of the molecular mechanisms mediating Bmi1 function in satellite cells

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The Polycomb group (PcG) protein Bmi1 is an epigenetic chromatin modifier involved in heritable gene repression and maintenance of stem cell self-renewal and progenitor proliferation. We recently showed that Bmi1 is expressed by the satellite cells (SCs) in adult skeletal muscle, and it is down regulated as the skeletal myoblasts differentiate. The loss of Bmi1 in mice results in a reduction in Pax7 positive satellite cells with reciprocal increase in MyoD positive cells and consequent reduction in muscle fibre size. Conversely, conditional activation of Bmi1 in mouse satellite cells increases their proliferation and capacity to re-enter the cell cycle upon stimulation in vitro without preventing their differentiation.

We analyzed the downstream mechanisms mediating the observed effects of loss and gain of function of Bmi1 in SCs. The expression of $p16^{Ink4a}$, $p19^{Arf}$ and $p21^{waf/cip1}$, cell cycle inhibitors known to be downstream effectors of Bmi1 was increased in the SCs isolated from Bmi1-/- mice. However, the expression of these genes was not downregulated in SCs overexpressing Bmi1 suggesting that other factors mediates regulate Bmi1 effect in this context.

Expression array analysis of SCs overexpressing Bmi1 reveals a significant upregulation of the metallothionein-1 (Mt-1) gene. Conversely, significantly reduced expression level of Mt-1 was found in SCs isolated from Bmi1-/- mice. Mt-1 is a cysteine-rich, low molecular weight protein, involved in metalloregulatory processes including cell survival and proliferation and it has significant antioxidant activity. Indeed, we found the expression level of genes involved in regulating intracellular redox homeostasis to be deregulated in these cultures. These data suggests that a role for Bmi1 in protecting SCs against antioxidative stress.

Poster 42

Does Bmi-1 over-expression increase myogenic satellite cells self-renewal capacity in ageing muscle?

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The Polycomb group (PcG) protein Bmi-1 is an epigenetic chromatin modifier involved in the maintenance of the self-renewal and proliferation potential in a number of stem cell populations. The loss of Bmi-1 results in cells becoming

senescent whereas overexpression increases the self-renewal ability of stem cells; these actions are at least in part mediated by the transcriptional repression of the INK4a/ARF locus.

Bmi-1 is expressed in the skeletal muscles satellite cells. In young mice, we show that conditional activation of Bmi-1 in satellite cells increases their proliferation and ability to re-enter the cell cycle but does not prevent them differentiating.

As satellite cells age, their function is compromised and this probably contributes to the poor regenerative capacity seen in aged muscle, although there is no significant reduction in the number of satellite cells.

Our hypothesis is that up regulation of Bmi1 in aged satellite cells can contribute to restoring their self-renewal capacity. To manipulate the expression levels of Bmi1 we have used a conditional Bmi1 transgenic mouse model: STOPFloxBmi-1. We have isolated pure satellite cell cultures from 12-18 month old single muscle fibres and activated Bmi1 overexpression by using an Adeno-Cre virus. We have analysed the satellite cells using a panel of satellite cell and myogenic markers (Bmi-1, Pax7, Myf5, MyoD, MyHC) 48hrs after infection with Adeno-Cre as compared to satellite cell cultures infected with Adeno-GFP virus. We have also analyzed the proliferation rate by BrdU uptake in Bmi-1 overexpression versus control satellite cells.

Muscle Channelopathies

Poster 43

Acetazolamide response in patients affected by Hypokalemic Periodic Paralysis

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Objectives: Hypokalaemic Periodic Paralysis (HypoPP) is a skeletal muscle channelopathy caused in up to 90% of cases by point mutations in either the skeletal muscle voltage-gated calcium channel gene CACNA1S, or the voltage gated sodium channel gene, SCN4A. Regular potassium intake often fails to prevent attacks. This has provided a rationale for the utilisation of additional drugs capable of preventing attacks, such as the carbonic anhydrase inhibitor Acetazolamide. Despite the beneficial effects of Acetazolamide, the mechanism of action is not understood and there are no randomised controlled trials of its use. We attempted to determine the proportion of patients who respond to Acetazolamide and if genotype influenced the efficacy of such treatment.

Methods: We searched the literature for all genotyped cases of HypoPP and documented any given response to Acetazolamide. We additionally reviewed the notes of all genotyped HypoPP patients who regularly attend our neuromuscular clinic and documented reported response to Acetazolamide.

Results: Approximately 50% of patients report benefit from Acetazolamide. There is a greater chance of benefit for those with a CACNA1S mutation. Glycine substitutions have the poorest outcome.

Conclusions: (i) Approxiamtely 50% of patients report no or detrimental response to Acetazolamide adding support to the need for randomised controlled trials and alternative therapies in HypoPP (ii) Genotype does appear to influence response to therapy highlighting the importance of access to a diagnostic genetic service (iii) The reported benefit generally described frequency and severity of attacks and there was little evidence regarding any influence on the development of myopathy.

Poster 44

The genetic skeletal muscle channelopathies: Genotype-Phenotype correlation and longitudinal studies

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The skeletal muscle channelopathies are rare Mendelian disorders caused by mutations in genes that encode ion channels. The non-dystrophic myotonias (NDMs) encompass a spectrum of disorders ranging from myotonia congenita (MC), to paramyotonia congenita (PMC) through to the potassium aggravated myotonias with muscle stiffness being the cardinal symptom. Mutations in either the muscle chloride or sodium channel underlie the NDMs. Andersen-Tawil syndrome (ATS) is characterized phenotypically by the triad of periodic paralysis, cardiac dysrhythmias and dysmorphic facies. Mutations in the potassium channel gene, *KCNJ2* are associated with ATS.

As members of the CINCH group (Consortium for the Clinical Investigation of Neurological Channelopathies), we at the MRC centre for Neuromuscular Diseases are involved in the first ever large scale multi-centre natural history trials of the non-dystrophic myotonias (NDM) and Andersen-Tawil syndrome (ATS). The aims of the trials are: to

characterize the phenotypic spectrum associated with specific genetic defects; collate data on disease progression and evaluate investigations used in diagnosis.

Our preliminary data suggest a number of key findings: the NDMs and ATS are associated with a wide phenotypic spectrum; they exhibit considerable genetic heterogeneity; the absence of mutations in a few individuals suggests that other new genes are also likely to underlie both disorders; and finally neurophysiological testing has an important role to play in diagnosis. The completion of these natural history trials will enhance our understanding of genotype-phenotype correlations, engender more accurate monitoring of the natural histories of NDM and ATS and allow us to assess endpoints for future treatment trials.

Poster 45

Double-blind placebo controlled cross-over study to investigate the efficacy of Mexiletine in patients with Non-dystrophic Myotonia in the UK

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Non-dystrophic myotonia (NDM) is characterised by muscle stiffness due to delayed relaxation of the muscle following voluntary contraction. At present there are no high quality randomised controlled trials investigating treatment for NDM. Mexiletine is a use-dependent sodium channel blocker. Anecdotal evidence and uncontrolled clinical trials have shown improvement in stiffness after administration. We are therefore looking at the efficacy of mexiletine in our NDM patients in a double-blinded placebo controlled cross-over study in collaboration with the CINCH (Clinical Investigation of Neurological Channelopathies) study group.

We aim to recruit a total of 15 patients in the UK with non-dystrophic myotonia as part of 60 patients recruited internationally at 6 centres. All patients will be randomised to a 4 week course of either mexiletine or placebo. This is followed by a one week wash out period and then a 4 week course of whichever drug the patient has not yet received. Patients are examined at the beginning and end of each treatment period.

The primary outcome measure is the patient's assessment of their stiffness recorded daily via an interactive voice response diary. Secondary outcome measures include the patient's assessment of weakness, pain and fatigue; quality of life measures with INQoL and SF-36 questionnaires; clinical myotonia assessment; quantitative assessment of grip myotonia and neurophysiology measuring CMAPs from long and short exercise tests and needle EMG.

This study aims to evaluate the efficacy of mexiletine in reducing stiffness in non-dystrophic myotonia.

Poster 46

Quantification of grip myotonia using a novel accelerometer device: a pilot study

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Background: Grip myotonia impedes voluntary hand-opening due to delayed muscle relaxation. Patients often describe this as muscle stiffness. Currently there are a limited number of objective measures to quantify myotonia which impedes the ability to choose reliable outcome measures for therapeutic trials.

Methods: We developed an accelerometer device which was attached to the right forearm and index finger. Subjects (patients with genetically confirmed myotonic disorder and healthy controls)were instructed to grip a peg with their right hand and on command fully extend their fingers as rapidly as possible. Time taken was measured by the accelerometer. Five successive grips/hand-openings were recorded over three trials on the same day to determine the validity and intra-rater reliability of the measurements.

Results: Intraclass coefficients of variation (ICC) to test for intra-rater reliability of the device were high. Good agreement between measures was found using Bland & Altman's method. A significant difference in hand-opening time was observed between the control and myotonia groups (P=0.015, F=9.07). Descriptive data trends illustrated different patterns of response in the subjects with different sub-groups of myotonic disorders.

Conclusion: Further studies with larger cohorts and methodological refinements are required but early data indicate the accelerometer device we have devised would be a useful and reliable tool to quantify myotonia. This could be used clinically to assess response to therapy and additionally as an outcome measure in therapeutic trials. If

reproducible patterns in the sub-groups of myotonic disorders are found in larger cohorts this could suggest a potential diagnostic use.

Myasthenia Gravis

Poster 47
Thymectomy - role in the management of myasthenia gravis
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Background: The role of thymectomy in the management of MG continues to be debated. In the absence of an adequate RCT, it is essential to review clinical experience.

Aim: To perform a 10 year audit of cases of thymectomy for MG in a tertiary referral centre.

Methods: Medical case notes of all patients who underwent thymectomy were retrospectively reviewed.

Results: 79 patients were identified. 22 were male. Average age at thymectomy was 32.5. Indications for thymectomy included: persistence of generalised symptoms (62%), bulbar symptoms (6%), a combination of both (8%), ocular MG (9%) and thymoma (15%). Over 90% were AChR ab positive. 45% had an enlarged gland on mediastinal imaging. Average length of ICU post-operatively was 2.2days. Immediate post thymectomy complications included pneuomonia (3.7%), pneumothorax (3.7%) and wound infection (2.5%). 7.5% suffered a hypertrophic scar. There were no long-term complications. Thymic histology revealed hyperplasia (47%), thymoma (22%), atrophic changes (10%) and a normal gland (21%). 77% of patients achieved an improvement in one point or more on the MGFA scale post thymectomy during follow up (6mths-2yrs). Conclusion: Thymectomy is commonly performed for MG and is associated with a sustained improvement in the majority of our patients.

Poster 48
Late recurrent thymoma: A case series
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Background: In patients with MG, thymoma may recur many years after initial resection
Aim: To describe cases of late recurrent thymoma amongst myasthenic patients in a tertiary referral centre
Methods: Cases of late recurrent thymoma were retrospectively identified and medical notes were reviewed

Results: 5 patients with a recurrent thymoma were identified. 4 were male. The average age at first tumour presentation was 47.6. 4 patients presented with MG. 4 had apparent full excision of their thymoma. One patient had invasive disease at first presentation with incomplete resection and adjuvant radiotherapy. The mean disease free interval to recurrence was 13.5 years. Three cases of recurrence were detected on routine scanning whereas the other 2 patients presented with cough or chest pain. Four patients had repeat surgery. Two patients had palliative radiotherapy and two had chemotherapy. 3 patients died. One patient was successfully treated for recurrence but developed sporadic motor neuron disease. One patient remains disease free 3 years after repeat surgery.

Conclusion: Thymoma may recur many years after surgery even if the initial tumour has apparently been completely resected. Tumour recurrence may be associated with a poor prognosis. Surveillance scanning after thymoma resection is essential and should continue indefinitely.

Poster 49
The use of stimulation jitter analysis with concentric needle electrodes in the diagnosis of myasthenia
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Objective The diagnosis of myasthenia in children, encompassing both Autoimmune Myasthenia Gravis and the congenital myasthenic syndromes, is challenging. We analysed our 12 year experience of using Stimulation **J**itter **A**nalysis by **C**oncentric needle **E**lectrode (Stim JACE) in the investigation of children with neuromuscular weakness and assessed its role in the diagnosis of myasthenia and other causes of neuromuscular transmission disorder (NTD).

Methods. Children who underwent Stim JACE between 1997 and 2009 were retrospectively identified from our departmental database. Medical records, pathology and genetic test results were reviewed. Sensitivity and specificity of Stim JACE were determined in the subgroup of patients with definite (genetic/antibody confirmed) and probable myasthenia (clinical diagnosis).

Results. A total of 151 Stim JACE fulfilled the study's entry criteria. The sensitivity of Stim JACE in the diagnosis of myasthenia varied from 93-97% with a specificity of 42-47%. Positive predictive value for diagnosis of myasthenia was 47-48% and negative predictive value 91-97%. In patients who had alternative diagnoses and abnormal jitter, the findings were often explained by the presence of a condition that was known to cause NTD, which could be diagnosed at the time of the examination. Other conditions previously not recognised as having NTD were also identified.

Conclusions. Stim JACE is a well tolerated, valuable and reliable tool in diagnosing myasthenic syndromes and other causes of NTD in children. We recommend the use of Stim JACE as an initial screening test in all children with symptoms suggestive of myasthenia.

Poster 50

Down-Regulation of ColQ by RNA Interference as a Potential Alternative Therapy in Myasthenic Disorders

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AChE is localised and concentrated at the NMJ by the anchoring protein ColQ. It functions to end signal transmission by cleaving the neurotransmitter ACh; either before it reaches the AChR or as it dissociates. Disorders of the NMJ may be either genetic or autoimmune, and often result in a loss of signal reaching the muscle. AChE is the target for one current therapy in myasthenia. AChE inhibitors aim to increase the amount of time ACh is present at the NMJ, enhancing neuromuscular transmission.

The aim is to investigate whether RNAi, targeting mouse *Colqi* mRNA, will enhance neuromuscular transmission; by causing a loss in the ColQ associated forms of AChE found specifically at the neuromuscular junction.

Three sequences, located at different positions along the mRNA length, were targeted for knockdown using siRNAs and shRNA-expressing vectors. The effects were investigated *in vitro* using an EGFP fusion construct and later *in vivo* using neuromuscular junction markers.
RNAi targeting mouse *Colqi* mRNA effectively down-regulates expression in both HEK TSA cells and at the NMJ of wild-type mice. Our future work will investigate whether the down-regulation of ColQ will be therapeutic in the AChR deficient congenital myasthenic syndrome mouse model.

Poster 51

Ephedrine treatment in *DOK7* CMS and investigation of potential mechanisms

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Recent reports suggest that patients with Dok-7 congenital myasthenic syndrome (CMS) benefit from treatment with ephedrine. We performed a prospective follow-up study to quantify the changes in patient muscle strength and mobility in response to ephedrine. Ten patients not previously on ephedrine, began treatment under supervision of 0.5-1.0 mg/kg/day and muscle strength was measured using the quantitative myasthenia gravis (QMG) severity score and mobility scores. Patients were assessed at initiation of treatment and at follow up at two months and 6-8 months. Ephedrine produced a progressive improvement in muscle strength with the median falling from 17/39 at baseline to 10/39 by 6-8 months ($p=0.009$, Wilcoxon signed-rank test). Similarly mobility scores showed a marked improvement ($p = 0.0006$, two tailed paired t test).

The mechanism through which ephedrine exerts this therapeutic effect is unclear, but it may be through an effect on □2-adrenergic receptors that partially compensates for the loss of Dok-7 function. We generated an immortalised human skeletal muscle cell line derived from a Dok-7 CMS patient in order to investigate possible affects on the AChR clustering pathway. This cell line demonstrated reduced agrin-induced AChR clustering compared to a control human muscle cell line, indicating that endogenous mutant Dok-7 affects either AChR cluster formation or cluster stability or both. However, initial experiments do not provide clear evidence for how ephedrine compensates for the mutant Dok-7.

Poster 95 (late submission)
Detection of AChR antibodies in 'Seronegative' Myasthenia Gravis and its clinical relevance illustrated in 2 cases
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Myasthenia Gravis (MG) is an autoimmune disease characterised by a defect in neuromuscular transmission. Patients with MG may test negative for both acetylcholine receptor (AChR) and muscle specific kinase (MuSK) antibodies on conventional immunoprecipitation assays, and are usually termed seronegative (SNMG). Their diagnosis can be difficult in the absence of autoantibodies and typical electrophysiological abnormalities. Consequently, there can be a delayed diagnosis, with significant implications in the clinical management. A recently developed cell-based assay (CBA) demonstrated the presence of low affinity IgG1 antibodies to clustered AChRs on transfected cell membranes in a cohort of SNMG patients.

Following an audit on the investigation of suspected MG in Addenbrooke's hospital, we identified two particular cases where the use of CBA was instrumental in the final diagnosis. In one patient who presented with generalized myasthenic symptoms, we were able to demonstrate the presence of IgG1 antibodies to clustered AChRs in sera that had been previously antibody negative on the radioimmunoprecipitation assay. The second patient, who had an atypical ocular MG and was previously misdiagnosed, was positive for AChR by CBA from the first sample, whereas the conventional assay showed positivity only in the sample taken 8 months later. In both cases an earlier diagnosis would have impacted on the patient's management and their quality of life.

These cases highlight the diagnostic challenges of SNMG, the potential of CBA in the early detection of autoantibodies and how this may further our understanding of the underlying autoimmune response.

Peripheral Nerve Disease

Poster 52
Exploring the experience of living with fatigue in people with Charcot-Marie-Tooth disease – a qualitative study
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This qualitative study explored the phenomenon of fatigue for people with Charcot-Marie-Tooth disease (CMT), while acknowledging the triggers, impact and strategies people have developed to manage this symptom in daily life.

Recent studies found that 67% of people with CMT report severe fatigue and investigation of the cause has found that there are different types of fatigue that may relate to separate mechanisms.

Methods: A qualitative, phenomenological approach was used to explore the experience of fatigue in people with CMT. Participants were recruited from the membership of the CMTUK support group. A total of 25 people participated in three focus groups. Group interviews were tape recorded and independently transcribed. Transcripts were coded and themes were identified.

Results: Four themes were identified:

1. triggers of fatigue: described as physical activity, stress, work and concentration.
2. description of fatigue: different types of fatigue identified, one related to physical activity and one that is independent of it.
3. impact of fatigue: frustration and difficulty balancing areas of life such as work, family, daily activities.
4. strategies to manage fatigue: planning, prioritisation and adaptations to conserve energy. Reports of reduced spontaneity, struggling and having to "make do".

Conclusion: This qualitative study supports quantitative investigations into fatigue with reports of different types. The analysis described triggers of different kinds of fatigue, the impact of managing to live with fatigue and the strategies people use to cope. The impact of fatigue on people's lives may require significant adjustment that could be facilitated by fatigue management approaches dealing with the wider activity and participation challenges that people with CMT face, not just the fatigue impairment.

Poster 53

Comparing gait performance of people with Charcot-Marie-Tooth disease who do and do not wear ankle foot orthoses Gita M Ramdharry (1,2); Alex Pollard (2); Jonathan F Marsden (3); Mary M Reilly (2). *1) St George's School of Physiotherapy Kingston University, Cranmer Terrace SW17 ORE,UK (2) MRC Centre for Neuromuscular Diseases, Department of Molecular Pathogenesis, Institute of Neurology, Queen Square, London WC1N 3BG, UK(3) School of Health Professions, University of Plymouth, Derriford Road PL6 8BH,UK.*

This study explores the differences in presentation and gait performance of a group of people with CMT (pwCMT) who wore AFOs and a group who did not. Relationships between gait performance and impairments were investigated.

Eleven subjects wore various types of AFO for daily mobility and 21 subjects did not. Primary measures of gait performance were measured using a 10m timed walk (comfortable and maximum speed) and a 6min walk test. Secondary measures included disease severity (CMT Examination Score); lower limb muscle strength (hand held dynamometry); sensory impairment; modified physiological cost index (PCI). Additional measures of fatigue severity and perceived walking ability were measured using the FSS and Walk-12 questionnaires.

Results: The AFO group had a slower maximum walking speed (t=4.794; P<0.000) and higher effort of walking (PCI t=-2.53; p=0.017). They also had greater disease severity (CMTES Z=-3.753; p<0.000), perceived greater walking difficulty (Walk-12 Z=-2.06; p=0.039) and were significantly weaker in the proximal and distal lower limb muscles. PwCMT who did not wear AFOs showed significant relationships between gait variables and lower limb muscle strength. The group who did wear AFOs showed significant relationships between gait variables and the Walk-12.

Conclusion: This analysis indicates that more severe pwCMT tend to use AFOs. Gait performance of pwCMT who don't use AFOs is determined by lower limb muscle function whereas gait performance of pwCMT who do use AFOs is determined by perception of walking difficulty. The support of the AFOs may reduce the impact of muscular function on gait performance, which could be influenced by more central factors.

Poster 54

Comparing activity levels between people with Charcot-Marie-Tooth disease and healthy controls – a pilot study

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This pilot study aims to compare physical activity levels, recorded using the SenseWear activity monitor (SAM), in people with Charcot-Marie-Tooth (pwCMT) and healthy matched controls. Correlations between SAM activity levels, self reported activity levels and impairments were investigated.

Twelve pwCMT and 12 healthy matched controls wore a SAM for the waking hours of 7 days. Primary comparisons of body mass index (BMI), calorie expenditure, energy expenditure (METs), time spent performing sedentary (<3 METs) and moderately vigorous (≥3 METs) activities were measured using the SAM in both groups, expressed as an individual's mean day. Secondary measures included reported activity levels (Phone-FITT questionnaire) and fatigue severity (FSS questionnaire) plus disease severity using the CMT Examination Score (CMTES).

Results: There were no significant group differences between calorie expenditure, energy expenditure, or time spent performing sedentary or moderate activities. Disease severity, self reported activity, and fatigue did not correlate with any of the SAM measures in pwCMT. No difference was seen in BMI (pwCMT mean BMI 25 ±3; Controls mean BMI 26 ±4), but both groups showed correlations between energy expenditure and BMI (pwCMT=0.61, P=0.035; Controls=0.61, P=0.031). PwCMT also showed a correlation between sedentary activity and BMI (0.64, P=0.027).

Conclusion: These early results indicate that pwCMT have levels of physical activity comparable with healthy controls. This contrasts with previous literature that reported pwCMT as an underactive population. The SAM and self reported measures of physical activity did not correlate, perhaps because the Phone-FITT questionnaire doesn't account for occupational activity. The correlation between BMI and activity variables raises general health

and well being implications. A larger trial will be required to see if the between group differences in physical activity are significant with more subjects.

Poster 55

Neurophysiological evidence for cerebellar dysfunction in neuropathic tremor

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Objective: To investigate if any evidence exists of cerebellar dysfunction in patients with immune-mediated neuropathy associated with tremor compared to those without tremor.

Background: The pathophysiology of neuropathic tremor remains largely unexplored. We aimed to establish whether there is any neurophysiological evidence of cerebellar dysfunction in patients with immune-mediated neuropathy associated with tremor compared to those without tremor, using the classic eyeblink conditioning paradigm. This paradigm at short intervals depends on the olivo-cerebellar circuit and does not require cerebral or basal ganglia structures.

Methods: Eyeblink conditioning was performed by pairing an auditory tone with a supraorbital nerve stimulus with delay interval of 400 ms in 3 age-matched groups: 8 patients with an immune-mediated neuropathy with tremor (5 CIDP, 2 MMNCB, 1 IgMPN), 6 patients without tremor (4 CIDP, 2 IgMPN) and 9 healthy controls. Conditioning consisted of seven learning blocks of 11 trials, followed by two extinction blocks.

Results: Comparing conditioned eyeblink responses (CRs) produced per block, patients with neuropathic tremor had significantly lower rates of CRs as the blocks progressed compared to neuropathy patients without tremor and healthy controls. Latencies of CRs, spontaneous blink rates and "alpha blinks" were not different between the three groups. Our results indicate an abnormality of associative learning in this group of patients with neuropathic tremor.

Conclusions: This study suggests functional changes in the olivo-cerebellar pathway of patients with immune-mediated neuropathy associated with tremor compared to those without tremor. Further work is required to determine if these changes are primary or secondary in nature.

Poster 56

A novel topical capsaicin model of “neuropathic pain” in human volunteers using cerebral evoked potentials and fMRI

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Capsaicin applied repeatedly to skin is effective in some neuropathic pain patients via desensitisation and degeneration of nerve fibres, and topical capsaicin has been used in human volunteer models of pain. We have addressed the need for a non-invasive and robust volunteer model with objective markers of “neuropathic pain” or hypersensitivity, particularly for the development of antagonists to TRPV1 (the heat and capsaicin receptor).

Topical capsaicin (1%) was applied to the forearm of 12 healthy volunteers for 15 minutes, and tests were performed prior to (baseline) and post application. Following capsaicin application: 1) there was a marked reduction in heat pain thresholds, but no change in warm detection threshold, suggesting selective sensitisation of nociceptors; 2) the amplitude of contact heat evoked A delta potentials was decreased, as would be observed in neuropathic patients; 3) the evoked pain scores (VAS) correlated negatively with the A delta amplitude, whereas they were correlated positively with pain at baseline, suggesting dysfunction of sensory fibres post-application; 4) fMRI showed pain-related BOLD activation areas were increased in size (including contralateral posterior cingulate gyrus, pre and post central gyrus, insula and the superior frontal gyrus).

The reduced heat pain sensory thresholds, decreased A delta potential amplitudes with a negative correlation with evoked pain scores, and increased area of activation in pain-related brain regions, in combination, are in accord with a “neuropathic pain” or hypersensitivity condition. Our protocol and markers may thus provide a useful model for the development of novel treatments for neuropathic pain, including TRPV1 antagonists.

Poster 57

Role of the Transcription Factor Sox-2 in the Control of Peripheral Nerve Development and Repair

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The efficient regeneration and repair of peripheral nerves following injury is absolutely dependent on the plasticity of Schwann cells. The ability of myelinated Schwann cells to return to an immature progenitor state is key to creating a permissive environment for axonal regrowth, consequent re-myelination and functional repair. Understanding these regenerative processes may have also have a huge impact upon our understanding of demyelinating conditions in the peripheral nervous system such as Charcot-Marie-Tooth or Guillain-Barre disease. A number of signalling mechanisms, such as c-Jun-N-terminal kinase (JNK)/c-Jun and Notch pathways, have been shown to drive Schwann cell de-differentiation both in vitro and in vivo. Our data suggests that the transcription factor Sox-2 may also play a key role in the plasticity of Schwann cells. We show that the transcription factor Sox-2 is expressed in immature Schwann cells and is rapidly re-expressed following peripheral nerve injury. We find that expression of Sox-2 in Schwann cells is regulated by both the JNK/c-Jun and Notch signalling pathways and experiments in Sox-2 null cells suggest that both pathways require Sox-2 function in order to repress myelin gene expression. Furthermore, we find that Sox-2 expression increases Schwann cell survival and is sufficient to regulate expression of molecules important for axon-Schwann cell interaction. The future identification of Sox-2 targets, together with an analysis of Sox-2 in vivo will further elucidate the role of this transcription factor in Schwann cell development and disease.

Poster 58

Anti-MA2 associated paraneoplastic myelo-radiculopathy

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Anti-MA2-associated paraneoplastic syndrome seen with germ cell tumours is almost always associated with limbic encephalitis. Here we describe a unique presentation of this syndrome in a 46 year old dentist, previously treated for seminoma with orchidectomy, who noticed weakness of pincer movement of the left hand whilst at work. Six weeks later he developed progressive finger-drop affecting the left hand over days and then right hand over weeks. Following nerve conduction studies and EMG showing reduced motor amplitudes and denervation without conduction block, a diagnosis of multifocal motor neuropathy with conduction block was considered and a trial of IVIG was given with no response. He then reported altered sensation spreading across his shoulders and back. Abnormal examination findings were restricted to his upper limbs with bilateral wasting of shoulder-girdle, arm and forearm muscles more marked distally, some fasciculations, but preserved reflexes and marked bilateral finger-drop. Mentation and general physical examination was normal. EMG again suggested a severe motor neuronopathy affecting the cervical regions. Detailed brain and spine neuroimaging was normal. PET scanning and testicular ultrasound were normal. Oligoclonal bands were positive in the CSF only. Anti-MA2 antibody titres were strongly positive. High dose steroids were given which slowed progression. A further orchidectomy was performed but histology revealed no tumour. His anti-MA2 antibodies fell after the orchidectiomy and he has remained neurologically stable since the surgery 4 months ago. Cervical myelo-radiculopathy in the absence of limbic encephalitis is a novel presentation of germ cell tumour-associated anti-MA2 paraneoplastic syndrome and highlights the expanding clinical spectrum seen with this antibody.

Poster 59

A novel mutation in the nerve-specific 5'UTR of the Cx32 gene causing CMTX1

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CMTX1 is the second most common cause of Charcot-Marie-Tooth disease (CMT), usually caused by mutations in the Cx32 gene. Cx32 has two tissue-specific promoters, P1 which is specific for liver and pancreas, and P2 which is nerve specific. Over 300 mutations have been described in the Cx32 gene, spread throughout the coding region. However, ~10% of CMTX1 families do not have mutations in the coding region. To date, five non-coding region mutations have been reported, three of which are located in the SOX10 binding site of the P2 promoter reducing activity of the promoter, and two located within the 5'UTR region.

We describe two families with X-linked inheritance and a phenotype consistent with CMTX1 who did not have mutations in the Cx32 coding region. Family 1 were also negative for mutations in PMP22, MPZ, SPTLC1 and GDAP1. Family 2 were negative for mutations in MPZ and rearrangements at chromosome 17p11.2. The non-coding region was sequenced and an upstream exon-splicing variant found at c. -373G>A which segregated with the disease in both families. This variant is located at the last base of the nerve-specific 5'UTR exon and thus may disrupt splicing of the nerve-specific transcript. Online consensus splice-site programs predict a reduced score for

the mutant sequence versus the normal sequence. Two other mutations have previously been described within the 5'UTR region, which created a potential donor splice site and were shown to prevent translation of mutant mRNA.

It is likely that other mutations within the Cx32 non-coding regions account for the CMTX1 families who do not have coding region mutations.

Poster 60

Variable severity of early onset CMT2 with compound heterozygous MFN2 mutations

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Mutations in Mitofusin 2 (MFN2) are the most common cause of axonal CMT (CMT2). Over 50 mutations have been reported so far, mainly causing autosomal dominant disease, though 6 families with recessive or semi-dominant inheritance have been described. We report three further families with index cases that are compound heterozygotes for two MFN2 mutations. All patients presented an early onset axonal neuropathy of variable severity. Their parents were non-consanguineous and had no signs or symptoms of peripheral neuropathy; all of them, except one (who was dead at the time of testing) were confirmed to carry the relevant heterozygous MFN2 variation. Multiplex ligation dependent probe amplification (MLPA) and DNA sequencing identified a deletion of MFN2 exons 7 and 8 and a previously reported missense mutation in both affected children in Family 1. MFN2 sequencing revealed two novel sequence changes, a nonsense mutation and a missense mutation, in Family 2 and a previously described missense mutation and a novel in-frame single codon deletion in three affected siblings in Family 3. Our findings confirm that MFN2 mutations can cause peripheral neuropathies with apparent recessive inheritance, although the parents in these families may be too young to show signs of neuropathy. Therefore a semi-dominant mechanism is possible. The findings also include the first report of an intragenic rearrangement of MFN2 (deletion of exons 7 and 8). Further work is required to establish the molecular mechanism underlying the pathogenesis of MFN2 mutations.

Poster 61

Diverse phenotypes are associated with missense mutations in the peripheral myelin protein 22 gene

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Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy and it includes a group of clinically and genetically heterogeneous disorders. CMT1A is the most common form of CMT and it is caused by a duplication of the 1.4 Mb region on chromosome 17 that contains the gene encoding the peripheral myelin protein 22 (PMP22). Point mutations in PMP22 are less common than the duplication and they can cause a variety of neuropathy phenotypes. We describe clinical, electrophysiological and molecular findings of eight families carrying PMP22 missense mutations. The phenotype was variable even within the same kinship and varied from mild hereditary neuropathy with liability to pressure palsies (HNPP) to severe CMT1. Neurophysiology showed variable upper limb motor nerve conduction velocity ranging from 2 to 48 m/s. Sequencing of the PMP22 gene identified six different point mutations: two families carried two novel mutations (Ser131Cys and Met69Arg), three families carried mutations that have been already reported and three families were found to harbour the controversial Thr118Met. The latter has been identified in heterozygous unaffected individuals as well as in patients affected by mild HNPP-like neuropathy. A clear correlation between the phenotype and the type and position of the aminoacid substitution has been not found. Although PMP22 point mutations are not frequently observed, our findings highlight the importance of sequencing PMP22 gene in patients with variable CMT phenotype.

Poster 62

Characterisation of novel mutations within HSP27 causing Charcot-Marie-Tooth Disease 2F and distal Hereditary Motor Neuropathy II

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Heat shock proteins (HSPs) are a highly conserved, ubiquitously expressed family of stress response proteins whose expression is increased in response to cellular stress. HSPs can function as molecular chaperones; facilitating protein folding, preventing protein aggregation, or targeting improperly folded proteins to specific degradative pathways. Heat shock protein 27 (HSP27) is a well characterised small HSP that plays a role in normal neuronal functions such as axonal growth and transport as well as cell survival; inhibiting apoptosis and protecting against oxidative stress. Recently, mutations have been discovered in the HSP27 gene that lead to both Charcot-Marie-Tooth Disease 2F and distal Hereditary Motor Neuropathy II (Houlden et al, 2008).

Initially, four HSP27 mutations were replicated by site-directed mutagenesis. The effects of transfection with each of the mutant HSP27 constructs as well as control, wildtype HSP27 on neuronal-like cells *in vitro* is currently being examined using SH-SY5Y cell lines. In the first instance we have examined the mutational effects on cell survival using LDH assays and aggregate formation using immunocytochemistry.

The aim of this study is to increase our understanding of the pathomechanism of the disease causing effects of HSP27 mutations. Since there is currently no effective treatment for dHMN, it is imperative that we increase our understanding of the disease mechanism in order to identify therapeutic targets for this disease. Preliminary findings will be presented.

Poster 63

C-Jun expression in human neuropathies: A pilot study

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Schwann cell dedifferentiation following nerve injury is important to permit neural survival and axonal regrowth. Animal studies have shown that c-Jun is a crucial regulator of Schwann cell plasticity, acting as a negative regulator of myelination, as well as promoting phagocytosis of myelin debris. It is hypothesised that c-Jun may also play a key role in human demyelinating neuropathies. This pilot immunohistochemical study is the first to examine the possible role of c-Jun in this context. We examined c-Jun expression in normal and diseased human sural nerves, as well as in myelinated dermal nerve fibres. Our study suggests that c-Jun is not expressed in normal dermal or sural nerve fibres, but is increased in pathological nerves. The key finding is that in dermal nerves, nuclear Schwann cell c-Jun is elevated in both inherited and acquired demyelinating neuropathies. Sural nerve c-Jun expression was also increased, but different patterns of staining were observed with different pathologies. The axonal and inherited demyelinating neuropathies were associated with increased c-Jun staining of either Schwann cell nuclei or axons. Nuclear c-Jun expression was not prominent in sural biopsies from acquired demyelinating disease, although c-Jun immunolabelling appeared sometimes to be associated with other compartments of the Schwann cells. Despite the small numbers of patients examined in this pilot study, it seems clear, first, that c-Jun is elevated in a number of nerve pathologies, and second, that this response varies between disease states. Further studies of c-Jun expression in human neuropathies may help clarify its role in these neuropathies and possibly offer new therapeutic targets in demyelinating neuropathies.

Poster 64

Genes for hereditary sensory and autonomic neuropathies: frequency in a UK series and genotype-phenotype correlations

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Hereditary sensory and autonomic neuropathies (HSAN) are clinically and genetically heterogeneous disorders characterized by sensory neuropathy, pain, ulcers and amputations, motor weakness later in the disease, axonal neuropathy on nerve conduction studies and axonal degeneration on nerve biopsy. To date, disease associated mutations have been identified in eight genes: two genes for autosomal dominant (SPTLC1 and RAB7) and six genes for autosomal recessive forms of HSAN (WNK1/HSN2, NTRK1, NGFB, CCT5 and IKBKAP). We analyzed a series of 95 patients with HSAN that were negative for the SPTLC1 gene. Mutation screening of the coding sequences of the WNK1/HSN2, NGFB, IKBKAP and FAM134B genes was carried out. We identified three disease-causing mutations in WNK1/HSN2, three in FAM134B and one in NGFB. Six mutations have not been previously reported and these were present as compound heterozygous or homozygous changes. The phenotypes associated with mutations typically consisted of an early-onset ulcero-mutilating sensory neuropathy and insensitivity to pain. There was clinical variability in and between genes in terms of clinical severity and age of onset. Overall disease-associated mutations were found in 8% of the studied patients although the RAB7, SPTLC1 (exons 1-4, 7-15),

CCT5 and NTRK1 genes are yet to be analyzed. Our genotype-phenotype correlation study broadens the spectrum of HSAN and provides additional insights for molecular and clinical diagnosis

Poster 65

Neuregulin-1 is required for remyelination and regeneration following injury to the adult peripaheral nervous system

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Neuregulin-1 (NRG1) is a protein expressed by axons which interacts with erbB2 and 3 receptors expressed on Schwann cells. NRG1 has a crucial role in axoglial signalling during development and modulates multiple aspects of Schwann cell development including the survival and migration of Schwann cell precursors and subsequently ensheathment and myelination of axons. The role of this molecule in adulthood and particularly following nerve injury has been unclear. We have used a mouse model to study this question.

Using Cre-Lox P technology *Nrg1* was conditionally ablated in a small subset (2-3%) of myelinated sensory and motor neurons which were labelled by Yellow Fluorescent Protein (YFP). In uninjured mice *Nrg1* deficient axons and the associated myelin sheath were normal. However following peripheral nerve injury, these axons showed either a thin or absent myelin sheath despite being ensheathed by Schwann cells. In addition *Nrg1* deficient axons were found to regenerate at a slower rate following sciatic nerve crush. NRG1 is therefore dispensable for maintenance of the myelin sheath however is essential for effective re-myelination and regeneration following peripheral nerve injury. It is an attractive target which may be modulated to enhance reparative processes following nerve injury.

Poster 66

A novel point mutation in the *Caenorhabditis elegans smn-1* gene provides a useful model for investigating Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA), an autosomal recessive genetic disorder, is characterised by the selective degeneration of lower motor neurons, leading to muscle wasting and in severe cases, paralysis and death. Deletions and point mutations lead to reduced expression of the widely expressed Survival of Motor Neuron (SMN) protein, which is implicated in a range of cellular processes including pre-mRNA splicing and axonal transport. The mechanisms underlying disease pathogenesis are unclear and there are currently no therapies that effectively treat SMA. Animal models developed to study SMN function include the nematode *Caenorhabditis elegans*. A single gene orthologous to SMN, *smn-1*, is present in the worm. A null *smn-1* mutant is presently available (Briese *et al.* 2009 *Human Mol Genet* 18:97-104) but as this leads to severe neuromuscular dysfunction and lethality, it is technically challenging to use for drug screening. We report the characterisation of *smn-1(cb131)*, an allele encoding a novel point mutation in a highly conserved region of exon 2, mimicking a missense mutation found in a patient with a less severe form of SMA. The *smn-1(cb131)* animals show mild, yet scorable, defects, much less severe than the knockout. Using an automated phenotyping system (Buckingham and Sattelle 2009 *BMC Neurosci* 10:84), mutants were found to swim more slowly than wild type worms. This model offers utility for screening chemical and RNAi libraries. Compounds ameliorating the swimming defect of *smn-1(cb131)* may accelerate the development of drugs for the treatment of SMA.

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Poster 67

Early presentation of Spinal Muscular Atrophy with Respiratory Distress (SMARD 1)

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SMARD1 is due to mutations in IGHMBP2 gene on Chromosome 11q13-q21. We report an infant with unusual early presentation. A baby was born at 35 weeks, breech, admitted to the neonatal unit for low birth weight. There were poor foetal movements throughout the pregnancy. The parents were consanguineous and had a previous small for dates still birth at term.

At two weeks of age he had a sudden respiratory collapse. Examination revealed axial hypotonia with good peripheral tone and antigravity movements. There was paradoxical breathing indicating diaphragmatic weakness.

After recurrent respiratory compromises he became ventilator dependent from 1 month of age. Later he developed contractures and urinary retention.

Neurophysiology; sensory and motor responses were absent and electromyography showed signs of denervation. Muscle biopsy was non-specific and other investigations including neuroimaging and SMN deletions were negative. He died after recurrent chest infections at 4 months of age. A mutation in the IGHMBP2 gene confirmed a diagnosis of SMARD1 after his death.

SMARD1 commonly presents between 6 weeks and 6 months of age in low birth weight, premature babies with early onset of respiratory compromise and ventilator dependence. There is predominently distal lower limb muscle weakness with involvement of the sensory and autonomic nervous system.

This is an unusual early presentation of SMARD at 2 weeks of age and the first reported case with urinary retention.

1. Severe infantile neuropathy with diaphragmatic weakness and its relationship to SMARD. Great Ormond street, Hospital for Neurology and Neurosurgery, London, UK (2003) Brain, Vol. 126, No. 12, 2682-2692, December 2003
2. Intensive Care Med. 2006 Nov; 32(11): 185-5. Epub 2006 Sep. 9 Milan, Italy
3. Clinical and Mutational Profile in Spinal Muscular Atrophy With Respiratory Distress (SMARD): Defining Novel Phenotypes Through Hierarchical Cluster Analysis HUMAN MUTATION 28(8), 808^815,2007
4. Interfamilial phenotypic heterogeneity in SMARD 1 Neuromuscular disorders journal November 2008 Grohmann K, Weinker TF, Saar K, et al Diaphragmatic SMARD is heterogeneous , and one form is linked to chromosome 11q13-q21Am J Hum Genet 1999;65(5):1459-62

Poster 68

Duloxetine for treating painful neuropathy or chronic pain; A Cochrane Systematic Review

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Duloxetine is a balanced serotonin and noradrenaline reuptake inhibitor licensed for the treatment of major depressive disorders, urinary stress incontinence and the management of neuropathic pain associated with diabetic peripheral neuropathy.

Objectives: To assess the benefits and harms of duloxetine for treating painful neuropathy and different types of chronic pain.

Methods: We systematically and comprehensively searched the medical literature from January 1966 to March 2009 for trials of duloxetine used for the treatment of painful peripheral neuropathy or chronic pain. We selected all randomised or quasi-randomised trials of any formulation of duloxetine, used for the treatment of painful peripheral neuropathy or chronic pain in adult participants. Two authors extracted and cross-checked data independently.

Results: Six trials (three of painful diabetic neuropathy and three of fibromyalgia) were identified including 2220 participants. Duloxetine at 60 mg daily is effective in treating painful diabetic peripheral neuropathy in the short-term risk ratio 1.65 (95% CI 1.34 to 2.03), NNT 6. Duloxetine at 60 mg daily is also effective in fibromyalgia over 12 weeks and 28 weeks. Adverse events were more common in the treatment arm with a dose dependent effect. Most side effects were minor, but 16% of participants stopped the drug due to side effects. Serious adverse events were rare.

Authors' conclusions: There is moderately strong evidence that duloxetine 60 mg and 120 mg daily are efficacious for treating pain in diabetic peripheral neuropathy and fibromyalgia but 20 mg daily is not. Minor side effects are common but serious side effects are rare.

Mitochondrial Disease

Poster 69

MRC Mitochondrial Cohort Study: Development of a UK Database

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Mitochondrial disease is a debilitating and often life-threatening condition that affects at least 1 in 5000 people in the UK. Clinical descriptions of the natural history of mitochondrial disease are largely anecdotal and there have been no systematic attempts to study disease progression in a tightly defined patient cohort.

The MRC has funded the Mitochondrial Cohort Study, a collaborative project involving the MRC Centre for Neuromuscular Diseases in London and Newcastle. It aims to create a national database of patients with genetically confirmed mitochondrial disease, make detailed clinico-pathological description of phenotype and correlate this with underlying genotype. The data will be crucial in providing accurate prognostic advice to patients and is a prerequisite for assessing efficacy of clinical interventions.

The cohort will help facilitate phase IIb and phase III drug trials and assessment of novel treatment strategies such as sequential resistance-endurance exercise. It will also provide the opportunity to assess various prevention strategies including those for cardiomyopathy, stroke-like episodes, migraine and epilepsy. The unprecedented access to family data including genotyping will also permit definitive studies on the transmission of mitochondrial DNA mutations and the effects of mitochondrial disease on female fertility and pregnancy.

Poster 70

Non-invasive Diagnosis of Single Deletion Disorders in Children with Suspected Mitochondrial Disease

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Background: Mitochondrial DNA (mtDNA) deletions may present in infancy with the Pearson marrow pancreas syndrome, or later in childhood or adult life with progressive external ophthalmoplegia (PEO), variably associated with myopathy and/or multisystem features including cardiac conduction defects, pigmentary retinopathy (RP), sensorineural hearing loss (SNHL) and endocrine disturbance. Traditionally, muscle biopsies have been used to diagnose patients presenting outside infancy. We report the diagnostic findings in 7 consecutive children presenting with single mtDNA deletions to our clinic in a 12 month period.

Cases and Methods: One child presented with transfusion-dependent sideroblastic anaemia and neutropaenia at 22 months; the remaining 6 had PEO, ranging in onset from 2 to 12 years and variably associated with poor growth, short stature, RP, SNHL, renal tubulopathy and endocrine disturbance. DNA was extracted from blood and urinary epithelial cells from all cases, and from muscle in 2 cases, to screen for mtDNA rearrangements and the m.3243A>G point mutation.

Results: Single mtDNA deletions were detected in blood in 5 of the 7 patients. Two patients had muscle biopsies, which showed a ragged red fibre myopathy in both cases, with isolated complex I deficiency in one case and normal respiratory chain enzymology in the other. Both had mtDNA deletions in muscle. In the 2 cases where mtDNA deletions were not detected in blood, they were present in DNA isolated from urinary epithelial cells.

Discussion: We propose that DNA should be extracted for analysis from blood and subsequently urine in children with suspected mitochondrial disease due to deletion of mtDNA, to avoid unnecessary muscle biopsies.

Poster 71

Development and validation of a mitochondrial disease-specific quality of life scale (Mito-QOL)

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Introduction: Mitochondrial diseases are a clinically diverse group of genetic disorders that affect organs heavily dependent on aerobic metabolism with extensive phenotypic and disease burden variability. Health related quality of life (HRQOL), is increasingly recognised as a fundamental patient-based outcome measure in bothclinical intervention and research. Generic outcome measures have been extensively validated in assessing HRQOL across populations and different disease states. However due to their inclusive construct it is acknowledged that not all relevant aspects of a specific illness may be captured. Hence there is a need to develop disease-specific HRQOL measures that centre on symptoms characteristic of a specific disease or condition. This study presents the conceptualisation, development and assessment of a valid and reliable mitochondrial disease-specific HRQOL measure (Mito-QOL).

Methods: Domain and item content validity of Mito-QOL was derived from semi-structured key-informant interviews with patients with mitochondrial disease and modified following piloting of the questionnaire by post. Items were eliminated with the use of standard psychometric criteria. Face to face interviews were conducted to verify comprehension and ensure validity. Construct validity was assessed by comparing Mito-QOL domain scores with similar domains of an established outcome measure (Short Form-36).
Results: Mito-QOL consists of 63 items within 16 unidimensional domains demonstrating excellent internal reliability (Cronbach's alpha $\geq$0.74) and construct validity.

Conclusion Mito-QOL is a valid and reliable disease-specific HRQOL measure which maybe suitable for use in clinical practice and research.

Poster 72

Uncovering the role of mitochondria in the pathogenesis 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. The histopathological hallmark of the disease is the presence within myofibers of "cores", areas devoid of mitochondria. The cause of the muscular weakness in core myopathies is unknown, as the presence of cores does not correlate well with clinical severity. While mitochondrial loss in cores strongly suggests their involvement in the pathophysiology, their function however has not been investigated in core myopathies. Calcium signals indeed regulate mitochondrial bioenergetic function, movement, biogenesis, and free radical generation. We hypothesize that altered calcium signalling in core myopathy patients negatively impact upon mitochondrial function, thus contributing to the muscle weakness. By confocal microscopy, we measured the mitochondrial membrane potential in myotubes from two patients and found that it was significantly decreased compared to controls. We quantified mitochondrial DNA copy number in myotubes in a variant of the disease, central core disease (CCD), and found that it was markedly increased. These results suggest that mitochodria are bioenergetically insufficient in core myopathy patients and contribute to the phenotype of muscle weakness. It appears that in CCD there is also an upregulation of mitochondrial biogenesis, presumably to compensate for the functional deficiency. Further studies are needed to better explain mitochondrial defects in core myopathy. This study provides novel insight into the mechanism of core formation and the disease pathogenesis.

Poster 73

OPA1 codes for a mitochondrial fusion protein found on the inner mitochondrial membrane

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Mutations in OPA1 are the most common cause of autosomal dominant optic atrophy (DOA), and until recently, this was thought to be a pure ocular disorder. By studying 104 patients from 45 independent families we have defined the clinical spectrum of this new multi-system mitochondrial disease, DOA+. We show that extra-ocular neurological and muscular complications affect ~20%, and include sensorineural deafness in childhood, followed by ataxia, proximal myopathy, peripheral neuropathy and progressive external ophthalmoplegia in the very late stages. We have also identified novel clinical presentations mimicking hereditary spastic paraplegia, and a multiple sclerosis-like illness resembling "Harding's disease", first described in patients Leber hereditary optic neuropathy. We show that the risk of multi-system neuromuscular disease is greatest in patients with mis-sense mutations, particularly those affecting the GTPase region of the protein, and that secondary mitochondrial DNA defects are likely to be responsible for the extra-ocular features. Individuals with DOA+ phenotypes also had significantly worse visual outcomes, and careful surveillance is therefore mandatory to optimise the detection and management of neuromuscular disability in a group of patients with already significant visual impairment.

Poster 74

Finding the Missing Gap – Mitochondrial DNA Deletions in Muscle Stem Cells

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Mitochondrial myopathies are a group of progressive muscle disorders caused by mutations in the mitochondrial genome (mtDNA). The culturing of muscle stem cells (satellite cells) has suggested that mutations may not be present in satellite cells from patients harbouring large-scale mtDNA deletions. The activation of satellite cells and

subsequent repair of muscle fibres, in the presence of heteroplasmy, may favourably shift the balance of deleted to wild type mtDNA, thereby decreasing mtDNA mutation load in affected muscle. However, the absence of mtDNA mutations in satellite cells needs to be confirmed, as previous investigations examining mtDNA deletions in these cells have focused on myoblasts, their cultured progeny.

We have used Fluorescently Activated Cell Sorting (FACS) using NCAM to isolate satellite cells for the analysis of mtDNA mutation load. An mtDNA deletion-specific SYBR Green real-time assay was designed to determine heteroplasmy levels for three patients harbouring mtDNA deletions. In all patients we detected the presence of deleted mtDNA in the satellite cells at percentages comparable to their muscle homogenate. The deletion was undetectable in the cultured myoblasts and myotubes from two of these patients, but was present in the samples from the third patient. These results were confirmed using a TaqMan real time PCR assay comparing the ND1 and ND4 regions of the mitochondrial genome.

These results suggest that mtDNA deletions are present in the satellite cells of our patients. However they may be lost in some cases upon stem cell activation and subsequent muscle repair. Potentially these techniques will allow us to determine exactly which patients will benefit from attempts to activate satellite cells in order to shift the balance of wild type to mutated mtDNA in muscle.

Poster 75

Infantile reversible COX deficiency myopathy caused by the m.14674T>C mutation in mt-tRNA^{Glu} in a German family

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Childhood-onset mitochondrial encephalomyopathies are usually severe, relentlessly progressive conditions, and have a fatal outcome. However, a puzzling infantile disorder, long known as “benign cytochrome *c* oxidase deficiency myopathy” is an exception because it shows spontaneous recovery if infants survive the first months of life. We have recently defined the principal molecular basis of the disorder by identifying a maternally inherited, homoplasmic m.14674T>C mt-tRNA^{Glu} mutation in 17 patients from 12 families. Here we describe the clinical presentation of this disease in 4 members of a German family homoplasmic for the m.14674T>C mutation in mt-tRNA^{Glu}. Although 10 maternal family members carry the homoplasmic mtDNA mutation, the clinical symptoms developed in only 4 of them in early childhood. The severity of symptoms was variable, but all affected patients showed a remarkable spontaneous recovery. We present the histological and biochemical findings in follow-up muscle biopsies of 2 affected brothers, confirming reversibility. Cell culture experiments for mt-tRNA^{Glu} steady state levels, mitochondrial translation and immunoblotting for mitochondrial proteins provide further evidence for a spontaneous recovery.
Early differential diagnosis between fatal and benign mitochondrial myopathies is of critical importance for prognosis and management of these infants, because the benign form is initially life threatening but ultimately reversible. Supportive care should not be withdrawn from these children early in life.

Poster 76

The m.3291 T>C mtDNA mutation causes Ekbom’s syndrome: expanding the clinical and genetic phenotype

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Since the first clinical description of this unusual mitochondrial eponymous syndrome by Ekbom in 1975, only a handful of cases have been described in the literature, with most patients associated with the MERRF phenotype and harbouring the m.8344 A>G mitochondrial DNA (mtDNA) point mutation.

We describe a patient with an Ekbom’s phenotype, with striking lingual and axial lipomatosis, cognitive slowing, deafness, diabetes, and neuromyopathy. Muscle biopsy showed a very abnormal appearance with dystrophic change, as well as numerous ragged-red and COX-deficient fibres. A full mitochondrial genetic work-up excluded known causes of this phenotype, with sequencing of the entire mitochondrial genome identifying a well characterised mutation m.3219 T>C in the *MTTL1* (tRNA $^{Leu(UUR)}$) gene, which has been previously associated with

the MELAS syndrome. To our knowledge, this is the first described case of a MELAS-associated mutation presenting with the Ekbom phenotype, highlighting the clinical heterogeneity that characterises mitochondrial genetic disease.

Poster 77

Complex I-deficient Leigh syndrome caused by a novel homozygous deletion in *NDUFS4*

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Background: Complex I deficiency, the most common cause of mitochondrial disorders, may present with heterogeneous clinical symptoms, including Leigh syndrome, a neurodegenerative disorder of infancy or childhood generally due to mutations in genes involved in mitochondrial energy metabolism. Although some complex I deficiencies have been associated with mitochondrial DNA mutations, the majority of complex I deficiency is probably caused by mutations in nuclear genes.

Methods: We performed whole genome SNP genotyping using 10K Affymetrix DNA chips in four patients with complex I deficiency and variable clinical features (Leigh syndrome, congenital lactic acidosis, cardiomyopathy). All 4 patients had consanguineous parents and were from the same geographical area; two patients were first cousins.

Results and Discussion: The gene encoding one known subunit of complex I, *NDUFS4*, was located in a region of homozygosity shared by 3 of the 4 patients. Sequence analysis of *NDUFS4* revealed a novel 8 bp frameshift deletion leading to a premature stop codon and thus predicting a truncated protein. This mutation was homozygous in a 6 month old girl with Leigh syndrome and isolated complex I deficiency, but heterozygous in her first cousin who had congenital lactic acidosis associated with deficiencies of both complexes I and IV. We suspect that this cousin has a different genetic basis of her mitochondrial disorder. The occurrence of two separate recessive disorders is not unusual in highly inbred pedigrees. *NDUFS4* sequence was wild type in the other 2 children, suggesting there are at least 2 causes of isolated complex I deficiency in this relatively small community.

Poster 78

Habitual physical activity in mitochondrial disease – do we need to intervene?

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Introduction: Rapid progress has been made in relation to our understanding of the molecular and genetic basis of many mitochondrial disorders. Yet despite this, and the recognition of the often devastating impact of mitochondrial disease upon daily life, avenues for therapeutic intervention are limited. Low levels of habitual physical activity have been recognised to have a strong negative relationship with muscle mitochondrial capacity, disease development and mortality. The aims of this study were to systematically assess habitual physical activity in a cohort of mitochondrial patients.

Methods: One hundred patients were enrolled in the study. Habitual physical activity was measured by a multi-sensor array and by completion of a self-report questionnaire. Disease severity was assessed using the Newcastle Mitochondrial Disability Adult Scale (NMDAS).

Results: Low levels of habitual physical activity were common. Seventy-eight percent of the patients achieved less than nationally advised levels of physical activity (10,000 steps per day) and almost half had an average daily energy expenditure of less than 1.4 METS. There were no systematic differences in physical activity as assessed by number of steps between different genotypes of mitochondrial disease using a one-way analysis of variance (p =0.78; ANOVA). Higher physical activity was associated with lower BMI and lower clinical disease burden. Forty-seven per cent of the patient group were overweight; 20% were obese.

Conclusion: Low levels of physical activity are prominent and constitute a significant and important modifiable risk factor in mitochondrial disease. These findings advocate the promotion of increased physical activity irrespective of genotype.

Poster 79

What modifies the clinical presentation of the common homozygous p.A467T *POLG* mutation?

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Over 100 different mutations have been identified in the mitochondrial polymerase gamma (*POLG*) gene in a wide range of mitochondrial diseases including Alpers syndrome, adult onset spinocerebellar ataxia, Parkinsonism and premature ovarian failure. Some mutations can behave as dominant or recessive alleles but autosomal recessive inheritance is much more common. The p.A467T mutation is one of the most commonly occurring mutations in the Caucasian population. Interestingly, not only compound heterozygous individuals, but also patients homozygous for p.A467T have a very variable clinical presentation, ranging from childhood-onset severe Alpers syndrome to adult-onset sensory axonal neuropathy with dysarthria and ophthalmoplegia (SANDO), indicating that additional factors must influence the expression of the clinical phenotype.

In this study, we summarize the clinical, histological, biochemical and molecular genetic data of 56 patients from 8 international mitochondrial diagnostic centers who are homozygous for the p.A467T *POLG* mutation. Thirteen patients presented with Alpers syndrome in childhood (< 15 years of age), 19 with adult-onset SANDO, while 24 patients showed a combination of epilepsy and ataxia. The ataxia was usually caused by the combination of cerebellar ataxia, described as mitochondrial spinocerebellar ataxia epilepsy (MSCAE) and sensory-axonal neuropathy with onset in the teens or early twenties. Interestingly, the clinical presentations tended to be similar in affected siblings indicating a modifying genetic factor. In an attempt to identify the genetic modifier, we have determined mtDNA haplotype, analyzed *POLG2, ANT1* and *PEO1*, and studied chromosome 15 haplotyping in 34 homozygous p.A467T patients. We correlate our results with the corresponding clinical phenotypes.

Inclusion Body Myositis and Myofibrillar Myopathies: IBM

Poster 80

Augmentation of the Heat Shock Response in an *in vitro* Model of Sporadic Inclusion Body Myositis

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Sporadic Inclusion Body Myositis (IBM), the commonest myopathy acquired by those aged over 50 years, remains without proven treatment. We developed an *in vitro* disease model, using primary satellite cells, in which over-expression of β-Amyloid Precursor Protein or exposure to inflammatory mediators IL1β/TNFα reproduced salient features of the cellular environment in IBM. With view to screening pharmaceutical agents, we investigated potential outcome measures relevant to IBM pathology, particularly those where inflammatory and degenerative processes might interact.

Using fluorescent imaging, elevated basal cytosolic calcium and disturbed ER calcium handling was demonstrated in myogenic cells over-expressing APP or exposed to IL1β/TNFα. These potentially pathological disturbances were significantly improved by treatment Arimoclomol, a co-inducer of the cytoprotective Heat Shock Response (HSR).

Cytoplasmic redistribution of TAR DNA-binding protein (TDP-43) from the nucleus is a fundamental feature of IBM tissue. Over-expression of APP and exposure to the inflammatory mediators reproduced this effect *in vitro*. These degenerative and inflammatory stimuli also triggered nuclear translocation of NfkB, reflecting its activation, a potential mechanism by which pathological processes are sustained in IBM. Redistribution of both TDP-43 and NFkB was significantly attenuated by Arimoclomol treatment. These data support further investigation of HSR augmentation as a therapeutic strategy in IBM.

Poster 81

Drug screening for new inhibitors of a human β-amyloid ($A\beta_{1-42}$) induced phenotype in a *Caenorhabditis elegans* transgenic line CL4176

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Alzheimer's disease (AD) and Inclusion Body Myositis (IBM) are highly prevalent diseases sharing the common histo-pathological feature of β-amyloid (Aβ) deposition in neurons and muscle cells respectively. Although the molecular trigger(s) behind amyloid formation and deposition in both diseases remain elusive, it is clear that Aβ acts as a cellular toxin that can trigger both neuronal and muscle degeneration. There is no cure for either AD or IBM and there is an urgent need for effective drug treatments. To this end, we have attempted to identify new candidate drugs that can suppress the adverse actions of Aβ in a *Caenorhabditis elegans* transgenic model (CL4176). Over-expressing the highly aggregation-prone Aβ peptide in body-wall muscle cells of these short lived nematodes is highly toxic to the cells resulting in a rapid paralysis phenotype. This provides a platform for rapid, *in vivo*, oral delivery, drug screening to identify compounds which can alleviate this paralysis phenotype. A library of Aβ aggregation inhibitors has been designed and synthesised by Senexis Ltd. We are currently examining approximately 300 of these small molecules for their ability to suppress amyloid toxicity and have already shown that RS-0406 and SEN1269 can significantly alleviate the harmful effect of Aβ. We anticipate that our approach may highlight novel compounds with potential therapeutic applications for Aβ associated diseases.
Supported by The Medical Research Council (UK) and Senexis Ltd, UK.

Poster 82

Heat shock protein induction as a therapeutic strategy for inclusion body myositis

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Inclusion body myositis (IBM) 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 IBM muscle. In particular, there is evidence for abnormal protein aggregation in IBM 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 aggregated proteins. Hsp upregulation 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 upregulating the HSR in an in vitro model of IBM. Using primary muscle cultures derived from neonatal rats we found that transfection with β-APP results in the formation of inclusions that are immunoreactive for ubiquitin, Hsp-70 and TDP-43 and which are associated with an increase in cytotoxicity. However, treatment with a pharmacological co-inducer of the HSR not only reduces inclusion formation but also increases cell survival. These results therefore suggest that targeting of the HSR may be of therapeutic benefit in IBM.

Poster 83

IBM-Net: a clinical database of inclusion body myositis patients

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Inclusion body myositis (IBM) is the commonest muscle disease beginning in those aged over 50. However it is poorly understood: its cause is unknown, it has no treatment and leads to progressive disability. Information on the pattern and prognosis of IBM is more based on anecdote from clinical experience, rather than firm fact.

Our project seeks to better characterise IBM by gathering data from as many cases as possible. This will build a valuable resource and form the starting-point for future studies.

Participants in the project will be seen at least annually and undergo a standardised assessment (information on the history of the illness, their medical background, how IBM affects everyday tasks and findings on physical examination). All data will be recorded on a secure central computer database (a modification of the existing Northstar package used for Duchenne dystrophy). To allow as many people as possible to participate in the study we will make data entry available (over a secure internet link) to other medical specialists around the UK, so people can be seen nearer to home and information entered locally. Alternatively, the necessary clinical data can be sent

to our hospital for us to enter into the database. By repeating our assessments over five years, we will be able to give a much more reliable and accurate prediction of the course of the disease.

As well as the clinical data, we also wish to ask participants to donate a small blood sample for storage and extraction of DNA. The blood and DNA samples will be stored and can be used in future studies of the disease.

Inclusion Body Myositis and Myofibrillar Myopathies: Myofilbrilar Myopathies

Poster 84

The Expanding Histopathological Profile of the Myofibrillar Myopathies

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Background: Myofibrillar myopathies (MFMs) are characterised by desmin protein aggregates and myofibrillar degeneration. Mutations in genes encoding extra-myofibrillar and myofibrillar proteins are well recognised, however, the genetic basis of many cases remains unclear. Despite advances in understanding of the pathogenesis of the MFMs, the precise mechanism by which they cause muscle disease at the molecular level remains elusive. We present a case which exemplifies both the clinical and the histopathological heterogeneity of this group of disorders.

Case history: The patient presented at the age of 30, following normal birth and early development, with predominantly proximal upper and distal lower limb weakness. Muscle biopsy was highly suggestive of an adult onset nemaline myopathy. Four further family members were subsequently found to have distal myopathies, 3 with cardiac involvement, and muscle biopsies suggestive of MFM. No mutation in ACTA1 was detected in our patient, and desmin mutations have been excluded in other family members.

Conclusion: This family presents with a dominantly inherited muscle disease with varied clinical and histopathological phenotypes. We are currently analysing further MFM genes to establish the common genetic defect suspected in all 4 family members. This case provides further evidence of the expanding histopathological profile of MFM.

Poster 85

Recurrent BAG3 gene mutation in a British family with two siblings with severe myofibrillar myopathy

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Myofibrillar myopathies (MFM) represent a heterogeneous group of neuromuscular disorders and have been associated with 55utosomal dominant mutations in the *DES, CRYAB, MYOT, ZASP, FLNC,* or *BAG3* genes. To date, BAG3-associated MFM has only been described in sporadic cases due to a *de novo* mutation (p.Pro209Leu). Here we report the first familial case of BAG3 associated MFM. The family, of British origin, consisted of two affected male siblings born from clinically unaffected and unrelated parents. The disease onset was at age 12 and 9 years respectively and was characterized by proximal muscle weakness, hypertrophic cardiomyopathy and progressive respiratory insufficiency. The younger brother died suddenly 4 months after the disease onset from a rapidly progressive restrictive/hypertrophic cardiomyopathy and respiratory insufficiency while the older had a cardiac transplant at age 14 years and is alive but severely disabled and ventilator dependent at the age of 29 years. Mutation analysis showed that both siblings were heterozygous for the p.Pro209Leu *BAG3* mutation, transmitted from the unaffected father who showed somatic mosaicism in blood cells for the detected variant. These observations suggest that BAG3-associated MFMs may also arise from spontaneous mutations occurring during early embryonic development in one of the parents, thus influencing transmission rate and recurrence risk in future pregnancies.

MRI in Neuromuscular Disease

Poster 86

Inter-Scan Reproducibility of Quantitative Neuromuscular MRI

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Background: If the potential of quantitative MRI (qMRI) methods to provide clinically-relevant indices of muscle pathology is to be realised, it is essential to determine their measurement reproducibility. Promising approaches include measuring fatty-infiltration with multi-point 'Dixon' methods and assessing inflammation and hydration through changes in the T2-relaxation time and the magnetization transfer ratio (MTR). We assessed the test-retest reproducibility of these methods in healthy controls.

Methods: Eight subjects, age 28.9±4.5 (mean±sd) yrs, were scanned twice at 3T with a 14 day interval between sessions 1 and 2. Acquisitions included mapping of dual-echo T2, MTR and 3-point Dixon fat-fraction. Lower limbs were imaged at the thigh and calf level using bony landmarks for accurate repositioning. For each measure, distribution histograms were generated for each limb and Gaussian functions fitted to the principle muscle signal peaks.

Results: Histogram peak positions (mean±sd) averaged over all subjects and scans were MTR: 37.1±2.0 p.u., T_2: 39.9±0.9ms and fat-fraction: 3.5±0.4%. Paired-sample t-tests between each of the peak position pairs were non-significant, indicating no systematic difference between scans 1 and 2. Intra-class correlation coefficients were 0.84 (MTR), 0.68 (T_2) and 0.33 (fat-fraction) and Bland-Altman coefficients of repeatability were 2.5 p.u., 1.5 ms and 0.9% respectively.

Conclusions: With careful experimental design at 3T these qMRI methods showed good inter-scan reproducibility in a homogenous group of healthy subjects. This will motivate future reproducibility studies in more pathologically-heterogenous patient populations, where qMRI measures offer considerable promise as markers of onset and progression.

Poster 87

Quantitative Magnetization Transfer MRI: A Potential New Source of Biomarkers in Skeletal Muscle?

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Background: The potential for MRI to provide quantitative biomarkers in neuromuscular disease is being increasingly recognized. The MRI magnetization transfer (MT) ratio has previously been shown sensitive to myopathy in e.g. LGMD and dermatomyositis. In this work we applied more rigorous quantitative MT (qMT) modeling methods to *in vivo* healthy skeletal muscle data for the first time. Our purpose was to evaluate the practicality of applying qMT in neuromuscular patients and to provide further physical insights into MT processes in muscle.

Methods: Lower limbs in 10 subjects were imaged using multiple 3D-FLASH MRI acquisitions at 3T with variable MT saturation pulse frequencies and amplitudes. The total acquisition time was less than 15 minutes. Data were analyzed using a 2-pool MT model previously established appropriate for data obtained in the CNS. T_1 and B_1 distributions were determined and incorporated in the qMT model, and parameters characterizing the 'free' and 'restricted' proton pools estimated in 7 different muscles.

Results: In the soleus muscle the average restricted-pool T_2-relaxation time was 5.9±0.2□s and the restricted-pool fraction was 8±1%, revealing the proportion of hydrogen moieties bound to macromolecules or constrained within hydration layers in this tissue; the specific underlying cellular or structural determinants, and pathological sensitivity of these quantities require further investigation. Similar values were obtained in other muscles. Muscle qMT may offer new markers of disease onset and progression in conditions such as myositis or the muscular dystrophies, obtainable with good precision within clinically acceptable scan times.

Poster 88

MRI in LGMD2I; a qualitative and quantitative analysis using the 3 point Dixon technique

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Limb Girdle Muscular Dystrophy 2I (LGMD2I) is caused by mutations in the fukutin related protein gene (FKRP) and associated with abnormal glycosylation of α-dystroglycan. MRI has been used for diagnostic purposes in muscular dystrophy, however more recently it has become of interest as outcome measure and therefore useful in clinical trials.The aim of this study was to (i) assess the pattern of fat infiltration in a large, multi-centre cohort of LGMD2I patients using a qualitative radiological score (ii) to implement a quantitative 3-point Dixon technique, developing a non-invasive tool to track the progression of fat infiltration longitudinally. We compared the infiltration with matched adult controls. From the qualitative T1w data, 182 muscles were assessed: over 50% were rated in the top two qualitative grades. There is widespread involvement of all muscle groups, however some are better preserved until late stage. The pattern of fat infiltration is consistent throughout male subjects with a typical whorled pattern of involvement in the posterior thigh muscles, particularly semimembranosus and a striated/variegated appearance in the soleus. From the quantitative Dixon data, there is overall correlation between qualitative and quantitative assessments ($\kappa = 0.79$, $p < 0.0005$), and more importantly the qualitative data correlated well with myometry and functional outcome measures. Fat infiltration in the vastus lateralis correlated strongly with knee extensor strength ($\kappa = 0.825$, $p < 0.001$) and the 'timed up and go' test ($\kappa = 0.901$). This study illustrates the usefulness of quantitative MRI as a potential outcome measure for muscular dystrophy trials.

Poster 89

An MRI biomarker for motor neuron disease?

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Background: Biomarkers are needed to improve diagnosis and therapeutic monitoring in MND, in which focality of onset and spread of disease are still poorly understood. Candidates must accurately reflect the inherent clinical and prognostic heterogeneity. Analysis of standard cerebral MRI sequences in MND have been disappointing to date. Diffusion tensor imaging (DTI) is a sensitive application for the non-invasive detection of white matter pathology, with promising biomarker potential. To date it has largely been applied to MND in a targeted way to areas of known pathology.

Methods: High-field cerebral DTI was applied in an unbiased whole-brain analysis across a group of 24 unselected heterogeneous MND patients, and well-matched healthy controls. Patients were scored for clinical upper motor neuron (UMN) involvement, and disability (using the revised ALS Functional Rating Scale, ALSFRS-R).

Results: A consistent reduction in fractional anisotropy (FA) was demonstrated in the central corpus callosum (CC), including correlation with disability. A separate whole-brain analysis in relation to UMN involvement highlighted the corticospinal tract (CST).

Interpretation: Corpus callosum FA is a promising biomarker for the heterogeneous syndrome of MND. It may also provide an important clue to the topographical spread of disease. FA reduction was related to disability, and did not simply reflect UMN involvement. The exquisite delineation of the CST in whole-brain analysis of FA correlated to clinical UMN involvement provided validation of both DTI and a 'bedside score' as markers of UMN neuronal damage in MND, and supports the inclusion of those subjects with apparently LMN/UMN-only phenotypes within one syndrome.

Poster 90

Magnetic Resonance Imaging and Sciatic Nerve Cross-sectional Area in Inherited and Inflammatory Neuropathies

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We describe radiological findings and measurements of the cross-sectional area of the sciatic nerve in a group of 10 patients with genetically-confirmed Charcot-Marie-Tooth disease type 1A (CMT1A), 9 patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and 10 healthy controls using magnetic resonance imaging. The mid-thigh of each individual was imaged using a short-tau- inversion recovery sequence and the nerve appearance radiologically evaluated with respect to the signal intensity and visibility of internal structure. The cross-sectional area of the sciatic nerve of each individual was measured by defining irregular enclosing regions of interest. The sciatic nerve area was enlarged in both pathologies compared to controls ($p<0.01$) with the greatest increases in CMT1A. Median (inter-quartile range) areas were 67.6(16.2)mm^2 for the CIDP group, 135.9(60.9) mm^2 for the CMT1A group and 43.3(26.6) mm^2 for the control group. Median nerve areas of both patient groups were

significantly larger than the controls (p<0.01). Logistic regression revealed that sciatic nerve area could be used to discriminate between patients and controls (p<0.04). Measurement of sciatic nerve hypertrophy on MRI may be of assistance in cases where the diagnosis is still in doubt, complementing other clinical investigations and potentially providing a tool to measure response to therapies.

Poster 91
Using MRI as a diagnostic tool in the skeletal muscle channelopathies
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MRI is increasingly used in neuromuscular disorders and there is emerging evidence that specific patterns of muscle involvement may provide useful diagnostic tools. The skeletal muscle channelopathies are a group of neuromuscular disorders which are all due to dysfunction of skeletal muscle ion channels with a subsequent abnormality of muscle membrane excitability. Although each sub group has its own causative gene(s) there are many overlapping clinical features and prioritising gene testing can prove difficult. All the muscle channelopathies are episodic disorders, the main symptoms being intermittent attacks of myotonia and muscle weakness but there are many reports of a permanent proximal myopathy developing in the muscle channelopathies. How common this is, whether it correlates with age, genotype, symptom frequency/severity or can be influenced by current therapy is not established.

We have performed MRI scans of the thigh and calf muscles of patients with genetically confirmed muscle channelopathies. To date all but one scan are abnormal. These and subsequent scans will be examined to determine if there is a universal abnormality and if so if there is a specific pattern or patterns of muscle involvement that may help to predict genotype.

If specific patterns emerge these may provide future diagnostic tools for the skeletal muscle channelopathies. In addition early data suggest despite the episodic nature of symptoms in the muscle channelopathies, the underlying disease mechanism is causing permanent muscle damage. This could be an important indication for early initiation of therapy as currently many patients manage symptoms by avoiding precipitating factors only.

This work was supported by the Brain Research Trust, Grant No. 5U54 RR019498-05 awarded by the National Center for Research Resources and an MRC Centre Grant.

Translational Research Tools

Poster 92
Registry of Outcome Measures (ROM); supporting review and selection of outcome measures (OMs) for studies and trials
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Objective: To provide accessible self-help tools and guidelines to support a systematic approach to review and selection of OMs for clinical trials/studies.

Background: Selecting the right OMs for clinical trials/studies is critical to success. Until done it can be a major barrier to translational research. The choice is best made by systematically reviewing existing OMs to identify suitable measures and inform decisions about adapting existing OMs or creating new ones. ROM helps this effort by offering information on an expanding number of potentially suitable OMs. We have added web-based tools to support the review and selection process.

Method: ROM incorporates:
1)a 'Tree of OMs' - allows the reviewer(s) to record OMs by category as being considered for a specific study or trial
2)a search engine that enables investigators to find potential OMs in ROM
3)a comparison table that displays information about multiple OMs to aid selection
4)a document in progress which will evolve to be a Manual for the review and selection of OMs

These web based tools are easily accessible to collaborative groups. They can be open access so that all investigators can see work in progress, avoid duplication of effort, and contribute their views.

Results: These tools have led to the publication of more OM records in ROM and are appreciated by investigators.
Conclusion: These new tools on www.researchrom.com will play an important part in helping translational research.

Poster 93
MRC NMD Biobank Service: An Overview
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The MRC Centre for Neuromuscular Diseases Biobank is a unique resource of human muscle, skin and nerve cultures available to the basic science community for a range of activities including the study of the pathophysiology of neuromuscular disorders, testing of novel therapeutic applications and the recruitment of patients in therapeutic trials.

Biopsy samples are predominantly collected from patients with confirmed or suspected diagnoses of Duchenne/Becker Muscular Dystrophy, congenital muscular dystrophies, RYR1 congenital myopathies and mitochondrial myopathies. Samples are taken when the patients are undergoing orthopaedic surgery or biopsy for diagnostic purposes. 'Control' samples are also collected from patients diagnosed with Cerebral Palsy or Adolescent Idiopathic Scoliosis when undergoing planned or elective orthopaedic surgery.

The Biobank has been actively collecting human biopsy samples since June 2008 from sites in London and Newcastle. The Biobank aims to collect 50 samples at each site in the first year, and 75 samples at each site every year thereafter. The Biobank has exceeded these collection targets.

Poster 94
Cochrane reviews: the best evidence for treating neuromuscular diseases?
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With emerging new therapies for muscle disease, such as corticosteroids for DMD and drugs that modify RNA processing, it is important that the most up to date systematic reviews of the literature are readily available to patients, clinicians and commissioners. The Cochrane neuromuscular review group is one of 51 editorial groups operating within the Cochrane colloquium. A Cochrane systematic review investigates a defined research question. The first stage is creation of a protocol which outlines the search strategy, type of study to be included, type of participants and methodological quality of trials to be evaluated. This process seeks to minimize bias and is peer reviewed prior to the initiation of the systematic review. Only randomized or quasi-randomised studies are included for meta-analysis. All completed reviews are peer reviewed once more prior to electronic publication and are then open to electronic criticism. All published reviews are updated regularly to ensure that available evidence is recent and unbiased. The processes involved in writing a systematic review will be outlined. A detailed summary of the neuromuscular review group's progress and future topics in muscle disease for review will be presented.

Clinical Trials in 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.

Open Trials

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

Status: Complete. Reporting stage
Sponsor: University College London
Funder: Muscular Dystrophy Campaign (MDC)
PI: Dr Mary Reilly

Charcot-Marie-Tooth disease 1A (CMT1A) is associated with a duplication of the peripheral myelin protein 22 (PMP22) gene. To date there is no pharmacological treatment for CMT1A patients. Treatments and therapy for CMT is restricted to symptomatic treatments such as physiotherapy and surgery for skeletal deformities. Recently, treatment with ascorbic acid (AA) has been shown to be effective for transgenic mice over-expressing PMP22, a model of the human disease. Treated animals had much less severe neuropathy as compared to untreated controls as shown by clinical and histological findings. Some clinical parameters even improved during treatment. This is a phase III prospective, multi-centre, randomised, double-blind, placebo-controlled study aiming to evaluate the efficacy of AA treatment in CMT1A. The study is now complete. Fifty participants were enrolled in the UK site at the National Hospital for Neurology and Neurosurgery.

For information about the study please contact Dr. Matilde Laura at m.laura@ion.ucl.ac.uk.

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

Status: Closed to recruitment
Sponsor: PTC Therapeutics
Funder: PTC Therapeutics
PIs: Prof. Francesco Muntoni, Prof. Kate Bushby

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder affecting young boys. The condition is disabling and life-threatening. A small subset of boys are classified as having Becker muscular dystrophy (BMD), a phenotypically milder form of the dystrophic muscle disease. In approximately 10 to 15% of boys with DMD and BMD the causative defect is the presence of a nonsense mutation in the dystrophin gene that truncates dystrophin protein production by introducing a premature stop codon into the dystrophin messenger ribonucleic acid (mRNA). PTC124 is a novel, orally bioavailable, small-molecule drug that promotes ribosomal read-through of mRNA containing a premature stop codon. Through this mechanism of action, PTC124 has the potential to overcome the genetic defect in boys for whom a nonsense mutation causes DMD/BMD. In vitro studies in cell lines with dystrophin nonsense mutations have shown that PTC124 can restore production of the missing dystrophin gene. This is an international, multi-centre, randomised, double-blind, placebo-controlled, dose-ranging, efficacy and safety study. The study's primary aim is to evaluate the effect of PTC124 on ambulation as assessed by the distance walked during a 6-minute walk test (6MWT). The double-blind arm of the study randomised 174 participants worldwide which are to be followed for a period of 12 months. At the completion of the blinded treatment, all compliant participants were eligible to receive open-label PTC124 in a separate extension study.

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

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 DYSTROPGY: A PHASE I/II CLINICAL TRIAL USING AVI-4658

Status: Completed. Closed to recruitment
Sponsor: Imperial College London
Funder: Department of Health (DoH)
PIs: Prof. Francesco Muntoni

The primary scope of the trial is to assess efficacy (dystrophin production) and safety of intramuscular administered morpholino oligomer directed against exon 51 (AVI – 4658 PMO). Antisense therapy with the use of antisense oligomers has the potential to restore effectively the production of dystrophin, the defective protein, in >70% of DMD. This could result in increased life expectancy through improved muscle survival and function. Recent scientific research has demonstrated the potential of this technique to skip mutated dystrophin exons, restore the reading frame and generate functional dystrophin protein. Having demonstrated proof-of-principle in human cell culture and animal model studies, we now intend to determine efficacy and safety of this approach to induce dystrophin exon skipping in children with DMD. This study is aimed at children with Duchenne muscular dystrophy above the age of 10 years with mutations than can be rescued by the skipping of exon 51 [45-50; 47-50; 48-50; 49-50; 50; 52; 52-63].

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

Status: Closed to recruitment
Sponsor: AVI Biopharma
Funder: Medical Research Council (MRC) and AVI Biopharma
PIs: Prof. Francesco Muntoni

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 is being conducted in London and Newcastle.

For information on the status of recruitment please contact Rahela Choudhury, Trials Coordinator (MRC centre London site) at r.choudhury@ich.ucl.ac.uk or Geoff Bell, Trials Coordinator (MRC centre Newcastle site) at geoff.bell@nuth.nhs.uk.

HYP HOP: DICHLORPHENAMIDE vs. PLACEBO FOR PERIODIC PARALYSIS

Status: Recruitment due to start April 2010
Sponsor: University Rochester
Funder: National Institutes of Health (NIH - USA)
PI: Prof. Michael Hanna

This is a phase III trial into Periodic Paralysis planned to start in 2009. 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.

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: Recruiting
Sponsor: University College London (UCL)
Funder: Food and Drug Administration (FDA – USA)
PI: Prof. Michael Hanna

The non-dystrophic myotonia (NDM) is a group of rare neuromuscular disorders that causes episodes of muscle stiffness (known as myotonias) and paralysis. Predominantly the muscles of the face, hands and legs are affected. In addition to these episodes a permanent and debilitating muscle weakness can develop. The optimal treatment for these disorders is unknown. Non-dystrophic myotonias are due to abnormalities of ion channels present in skeletal muscle membranes. There is experimental evidence that drugs like mexiletine which block the abnormal function of these ion channels allow the muscle to perform normally. The study aims to test the efficacy of mexiletine in the treatment of the non-dystrophic myotonias. This proposal involves a multi-centre, double-blind, placebo-controlled cross over trial of total duration nine weeks. Approximately fifteen participants will be enrolled in the UK at the National Hospital for Neurology and Neurosurgery.

For information on the status of recruitment please contact Dr. Dipa Raja Rayan at d.rajarayan@ion.ucl.ac.uk 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: Recruitment due to start soon
Sponsor: University College London (UCL)
Funder: Medical Research Council (MRC)
PI: Prof. Michael Hanna

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. Adrian Miller at a.miller@ion.ucl.ac.uk or Gisela Barreto, Trials Coordinator at gisela.barreto@uclh.nhs.uk.

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 Michael Hanna

Andersen-Tawil Syndrome (ATS) is a rare form of periodic paralysis that is associated with serious heart-rhythm abnormalities. ATS is characterized by a triad of episodic muscle weakness, long-QT syndrome with potentially

fatal cardiac dysrhythmias and skeletal developmental anomalies. The underlying cause of this potentially fatal condition is only partly understood and there are no established treatments. Mutations in the KCNJ2 gene encoding Kir2.1, an inward-rectifying potassium channel account for approximately 60% of ATS cases (termed ATS1), the remaining 40% are presumed to have an as yet undetermined gene lesion and are designated ATS2. ATS1 and ATS2 are phenotypically indistinguishable. The treatment of ATS has been largely anecdotal and empirical. This proposal involves a multi-centre, placebo-controlled 'n of 1' study design of total duration 45 weeks. The expected total enrolment for this multi-centre study is 16 participants. The aim of this study is to determine whether potassium supplements and/or acetazolamide alter the duration of muscle weakness and potentially life-threatening heart rhythm abnormalities in patients with ATS.

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

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: Open to recruitment
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 j.spillane@ion.ucl.ac.uk or Natalie James at natalie.james@uclh.nhs.uk.

DMD HEART PROTECTION TRIAL

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

Status: Set-up phase
Sponsor: Newcastle NHS Foundation
Planned start date: 2010
Funder: British Heart Foundation
PI: Prof. Francesco Muntoni

Duchenne muscular dystrophy [DMD] is an X-linked recessively inherited neuromuscular disorder due to a deficiency in the expression of the protein dystrophin on the inner aspect of cell sarcolemma. Its clinical course has traditionally been characterised by progressive weakness of proximal limb-girdle muscles and calf muscle hypertrophy.Duchenne-affected individuals typically lose ambulation and become wheelchair-dependent before the age of 13 and die from cardio-respiratory failure at around the age of 20 years. From the cardiology perspective, some 90% of males with DMD develop a severe, progressive form of cardiomyopathy. Twenty to 30% have evidence of left ventricular impairment on echocardiography by age 10 years. Abnormalities in left ventricular

function are evident in an even larger proportion of patients at all ages when more sensitive imaging techniques, such as tissue Doppler, magnetic resonance or metabolic imaging, are deployed. Despite the severity of cardiac involvement in DMD, cardiologists have largely ignored this particular inherited form of cardiomyopathy. This is due to the fact that, because of their inability to exercise, cardiac symptoms only occur terminally in DMD patients when all cardiac reserve has been eroded. Even today in most hospitals, cardio-active drug therapy is only started in patients with DMD when overt heart failure is evident and, even then, is typically deployed tentatively for symptom control, without any expectation that it can prolong life. The objective of this trial is to determine whether the introduction of ACE inhibitor combined with beta-blocker therapy, before the onset of echo-detectable left ventricular dysfunction, can delay the age of onset and/or slow the rate of progression of cardiomyopathy compared to placebo in males with DMD. This is a double-blind randomised, placebo-controlled Phase III trial of combined ACEinhibitor and beta-blocker therapy (perindopril and bisoprolol) over a minimum of three years and a maximum of five years. 140 participants (70 per arm) are to be enrolled and randomised.

For more information about the study please contact trial coordinator Rahela Choudhury at r.choudhury@ich.ucl.ac.uk.

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

Status: REC Approval pending
Sponsor/Funder: GlaxoSmithKline
PI: Prof Dimitri Kullmann

Myasthenia Gravis (MG) is the best understood autoimmune disease (a disease in which the immune system attacks some part of the body). This attack is directed by various parts of the immune system. There is a continued search for newer drugs that will be of benefit in the treatment of MG. There is a continued search for newer drugs that will be of benefit in the treatment of MG. Otelixizumab has been identified as a possible treatment for MG. However before clinical trials can be considered additional information is needed to determine how it interacts with the immune system of patients with MG. In this study adult patients with MG will be invited to provide blood samples (50 ml) for research purposes. Blood collected from patients will be used for Tcell assay and autoantibody assay development. Patients may be asked to provide a repeat blood sample (additional 50ml) after 46 months following the initial collection to see if T cell activation changes over time. Up to 40 participants will be enrolled overall across two participants centres: one in the US and the other in the UK.

The study is being sponsored by GlaxoSmithKline group of companies.

Natural History – Longitudinal Studies

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. Michael Hanna

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. Emma Matthews at d.rajarayan@ion.ucl.ac.uk.

EPISODIC ATAXIA SYNDROME: GENOTYPE-PHENOTYPE CORRELATION AND LONGITUDINAL STUDY

Status: Recruiting
Sponsor: University College London
Funder: National Institutes of Health (NIH – USA)
PI: Prof. Michael Hanna

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: Recruitment to start soon
Sponsor: University College London Hospitals
Funder: National Institutes of Health (NIH – USA)
PI: Dr. Mary Reilly/Prof. Francesco Muntoni

Charcot-Marie-Tooth Disease (CMT) and related disorders (distal hereditary motor neuropathy (dHMN)and hereditary sensory and autonomic neropathy (HSAN)) are a clinically and genetically heterogenous group of disorders affecting approximately 1 in 2500 people. People with this condition present with upper and lower limb weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles. Despite the clinical similarities among patients with CMT the group is genetically heterogeneous. Advances have been made in identifying the genes that cause CMT and the molecular organisation of the peripheral nervous system (PNS) nevertheless the optimal management and treatment of the different variants of this disorder is not known and moreover natural history data is lacking for most forms of inherited neuropathies. This is a 5 year study that will be conducted by four centres in United States and two centre in the UK (National Hospital for Neurology and Neurosurgery and Great Ormond Street Hospital). The aim of the project is to fully characterise the features of different types of CMT and the longitudinal progression of the disease. The data will also be used to establish clinical relevant endpoints for use in therapeutic trials. The identification and genetic characterisation of patients will facilitate the recruitment of participants for future therapeutic trials. Ultimately the information gained with this study will lead to the improvement in the treatment and management of CMT. The study is also seeking to establish an appropriate paediatric impairment scoring method for CMT and establish a database for the inherited neuropathies. The study will include both adult and paediatric patients. Evaluations will consist of a neurological history and examination, nerve conduction velocity (NCV) study and in some selected cases skin biopsy. This is a NIH funded study. Fifty patients will be enrolled at the National Hospital for Neurology and Great Ormond Street Hospital.

For more information about the study please contact Dr. Matilde Laura at m.laura@ion.ucl.ac.uk.

OUTCOME MEASURES IN SMA TYPE II AND II

Status: Set-up phase
Funder: SMA Europe
PI: Prof. Francesco 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 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.

PERIPHERAL NEUROPATHY OUTCOME MEASURES STANDARDISATION STUDY (PERINOMS)

Status: Set-up phase
Sponsor: Erasmus Medical Center
PI: Dr Michael Lunn

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.

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. Michael Hanna

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

For information on the status of recruitment please contact Dr. Sanjeev Rajakulendran at s.rajakulendran@ion.ucl.ac.uk.

Exercise Studies

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

Status: Open to recruitment
Sponsor: University College London Hospitals
Funder: Muscular Dystrophy Campaign (MDC)
PI: Dr. Mary Reilly

Charcot-Marie-Tooth (CMT) disease is a form of hereditary peripheral neuropathy. People with CMT present with weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles. The aim of this study will be to investigate the efficacy of a 16 week home based programme of training to increase hip flexor muscle strength and walking endurance. Additional measures of gait speed, exertion, fatigue, disability and general activity will also be recorded. Baseline impairment measures will be obtained to ascertain predictors of strength gains. This study will use a single blinded, randomised cross over design to investigate if training the hip flexor muscles will strengthen the hip flexor muscle and improve walking endurance in people with all types of CMT.

The trial will included 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.

For information about recruitment contact Alex Pollard, Research Physiotherapist at a.pollard@ion.ucl.ac.uk.

EXERCISE TRAINING IN PATIENTS WITH MITOCHONDRIAL DISEASE: ASSESSING THE BENEFITS

Status: Recruiting
Sponsor: University Newcastle
Funder: Muscular Dystrophy Campaign (MDC)
PI: Prof. Doug Turnbull

Mitochondrial myopathies are a very important group of muscle diseases associated with weakness, pain and fatigue. At present, treatment options are very limited. Exercise therapy has been found to have some benefit in this group of patients and we wish to explore this further in terms of both strength and endurance. The aim of this study is to demonstrate that strength exercise training is an effective approach to therapy in certain patients with mitochondrial myopathy, specifically those with sporadic mutations in mitochondrial DNA. Based on our previous research studies, we believe that such training will improve muscle strength, mitochondrial function, exercise tolerance and overall quality of life.

The main objectives will be:
To confirm that endurance training in patients with mitochondrial abnormalities improves quality of life, exercise tolerance and oxidative capacity.
To determine the ability of resistance muscle strength training to improve skeletal muscle strength and oxidative capacity by incorporation of satellite cells into mature myofibres.

Participants are expected to commit to an exercise training and testing over a period of 4 to 8 months. The study will include patients between the ages of 18 and 65 years who have had a previous muscle biopsy showing a defect in skeletal muscle mitochondrial DNA that is either in the form of a sporadic point mutation or single large-scale deletion. Patients who have this type of mutation and do not have any family members that are affected and have no major cardiac involvement, hypertension, pulmonary or peripheral vascular disease that may complicate findings.

For information about recruitment contact Geoff Bell at geoff.bell@nuth.nhs.uk or Caroline Hodgson at c.hodgson@ncl.ac.uk.

Imaging Studies

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. Tarek Yousry/Dr John Thornton

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) will be recruited and 40 patients with genetically confirmed Charcot Marie Tooth Disease (CMT) will be recruited. In addition to the two patient cohorts, two groups of healthy volunteers each of size 40 will act as comparators for the disease groups. Each of the patients enrolled in the study will undergo an MRI scanning session in which the quantitative MR techniques developed in Phase 1 with the health volunteers will be applied. In addition to the MRI scanning sessions, each patient will undergo a clinical examination to record the main clinical features of their disease status including an electrophysiological nerve conduction assessment. In the final phase of the study, a sub-group of the patients will then be followed-up at 6 month intervals for 5 years in a longitudinal natural history study of IBM and CMT that focuses on the MR methods and clinical findings that were shown to be most illuminating. Changes over time in the MRI parameters in the diseased groups and Healthy volunteers will be compared.

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

Secondary objectives of the study include:
-The development of novel quantitative MR techniques for targeted assessment of the human neuromuscular system
-To more fully characterise both the magnetic resonance imaging and clinical features of inclusion body myositis or Charcot Marie Tooth disease as compared with healthy individuals and to study the progression of these characteristics with time over a period of 5 years.

For more information about the study please contact Dr Jasper Morrow at j.morrow@ion.ucl.ac.uk.

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