Muscular Dystrophy UK
Fighting muscle-wasting conditions

MRC Centre for Neuromuscular Diseases

Eighth UK Neuromuscular Translational Research Conference

19th – 20th March 2015

Centre for Life
Times Square
Newcastle upon Tyne


MRC
Centre for
Neuromuscular Diseases


Newcastle
University


UCL


Muscular
Dystrophy UK
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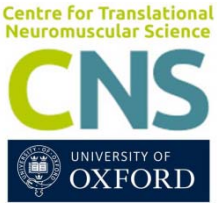
Centre for Translational
Neuromuscular Science
CNS
UNIVERSITY OF
OXFORD


wellcome trust centre for
Mitochondrial Research


MRC
Mitochondrial
Biology Unit


MRC
Functional
Genomics
Unit


NHS
National Institute for
Health Research


cbav
centre for brain ageing and vitality

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Welcome to the eighth annual meeting of the MRC Centre for Translational Research in Neuromuscular Diseases

UK Neuromuscular Translational Research Conference Newcastle 2015

Dear Colleagues,

We are delighted to welcome you to Newcastle for the eighth annual UK Neuromuscular Translational Research Conference. We are very pleased that this conference continues to be jointly hosted by the MRC Centre for Neuromuscular Diseases and Muscular Dystrophy UK. In addition, this year we have worked closely with colleagues in the London-Newcastle MRC Centre steering committee, the MRC Mitochondrial Biology Unit in Cambridge, the Wellcome Trust Centre for Mitochondrial Research in Newcastle and the MRC Functional Genomics Unit in Oxford to develop the scientific translational research programme.

Major translational themes this year include mitochondrial disease, genomic data and its use in support of translational research for neuromuscular diseases, the identification and validation of new targets in neuromuscular diseases and the increasing role of MRI biomarkers for diagnosis and disease/therapy monitoring.

We are delighted to welcome a world-leading neuromuscular clinician scientist who will deliver the conference named lecture; Professor Carsten Bonnemann, Senior Investigator and Chief of the Neuromuscular and Neurogenetic Disorders of Childhood section, NIH, USA, will give the third John Walton Lecture (dedicated this year to the memory of David Gardner-Medwin).

The mission of the MRC Centre is to translate science into new experimental medicine trials and find treatments for children and adults with serious muscle wasting diseases. Collaborative working and interdisciplinarity represent a fundamental platform for successful translation. The MRC Centre works to bring together clinicians, scientists, patient organisations and patients in order to advance the UK translational research effort. This is a particularly exciting time in the field as scientific discoveries are revealing an increasing number of tractable therapeutic targets.

The MRC Centre continues to work closely with all its partners to support the development of a clinical trials culture and aims to embed an experimental clinical trials network in neuromuscular clinical practice. We will continue to work hard to form effective research and clinical links with as many other UK neuromuscular groups as possible. In the renewed Centre (renewed 2013-2018) we have developed strong experimental medicine links with, and received important coordinated support from, three NHS NIHR Biomedical Research Centres based at University College London Hospitals, Great Ormond Street and at Newcastle University Hospitals. The MRC Centre is also pleased to contribute to the NIHR National Rare Diseases Translational Research Collaboration.

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 Newcastle University and was renewed for a further five years in 2013. The Centre continues to be closely linked to its partner NHS organisations, University College London Hospitals NHS Foundation Trust, Great Ormond Street Hospital for Children NHS Foundation Trust and Newcastle upon Tyne Hospitals NHS Foundation Trust. The Centre has also developed strong links with groups in Oxford and Cambridge which we will develop further in this current phase of the Centre.

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

We have received over 120 high quality abstracts, and there will be platform presentations and dedicated poster sessions each day as well as guided poster discussions. This year we have also introduced for the first time a poster flash session where abstracts are selected to be presented in 5 minute slots using only three slides. We hope this will prove a popular session. There will be five £500 poster prizes for young investigators. Accepted abstracts will be published in a special supplement of the journal *Neuromuscular Disorders* with Professor Mary M. Reilly as the special guest editor.

This year we were have also introduced a patient day which takes place the day before the conference.

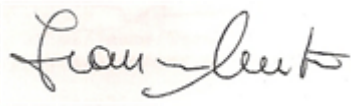
As the Centre Director, I would very much like to thank Professor Mary M. Reilly who has lead the joint MRC-Muscular Dystrophy UK meeting scientific planning team: Professors Kate Bushby, Doug Turnbull, Francesco Muntoni, Dame Kay E. Davies, and Dr Marita Pohlschmidt. I also sincerely thank Christine Oldfield and Marita Pohlschmidt for their very hard work in organizing this meeting. I am very grateful for the interest, sponsorship and support of industry colleagues who are working with us to develop new therapies for patients with neuromuscular diseases.

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, productive and entertaining two days in Newcastle.

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

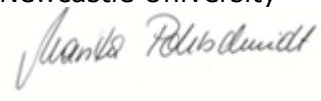
Professor Mary M. Reilly
Co-Director
MRC Centre for Neuromuscular Diseases
UCL Institute of Child Health



Professor Francesco Muntoni
Co-Director, ICH/GOS
MRC Centre for Neuromuscular Diseases
UCL Institute of Neurology

Professor Katie Bushby
Co-Director, Newcastle
MRC Centre for Neuromuscular Diseases,
Newcastle University

Professor Doug Turnbull
Co-Director
MRC Centre for Neuromuscular Diseases
Newcastle University



Dr Marita Pohlschmidt
Director of Research,
Muscular Dystrophy UK

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

Welcome from Robert Meadowcroft – Chief Executive of Muscular Dystrophy UK

I am writing on behalf of Muscular Dystrophy UK to extend a very warm welcome to the 2015 UK Neuromuscular Translational Research Conference organised in partnership between the MRC Centre for Neuromuscular Diseases and Muscular Dystrophy UK (formerly known as the Muscular Dystrophy Campaign).

Muscular Dystrophy UK has supported this important meeting since its inception and we are delighted that scientists and clinical researchers from across the field of neuromuscular disorders have this opportunity to come together to showcase progress in the field.

Indeed, many advances have been made through research into neuromuscular disorders backed by the charity's research funding over more than 50 years. During this time our families and supporters have raised more than £55 million to fund cutting-edge science and research, whilst a further £50 million of the charity's funds has been invested in care and support for families.

I am pleased to say our research programme is expanding and we are now working in partnership with a number of national and international charities to continue funding high quality research. As more genes are identified and a patient's genetic diagnosis is more precise, families today often decide to restrict their donations to research in a specific condition and the charity has established new mechanisms to support them. Last year saw the first international call made by the charity for research into a single condition, Ullrich congenital muscular dystrophy, and we are very pleased this new partnership involves four leading charities from France, Ireland, Switzerland and the USA.

We are encouraged by the growing number of clinical trials taking place and delighted that potential treatments are now on the horizon for a number of conditions. ***Translarna***, the first drug for Duchenne muscular dystrophy addressing the underlying genetic cause has received regulatory approval from the EMA while Parliament has recently voted in favour of regulations to permit the first clinical trials using an IVF technique for ***mitochondrial donation.*** It is crucial for families to have access to emerging drugs and treatments as swiftly as possible and our ***Fast Forward*** campaign presses for faster access, investment in clinical trials and infrastructure and support for comprehensive and consistent care and treatment.

I must also thank all our clinical colleagues who give their time to work closely with us to improve and extend specialist care for all those with muscle wasting conditions. As well as the impressive progress in research, significant improvements in clinical care have been achieved as a result of a collaborative effort over many years and I am delighted this progress is accelerating. Muscular Dystrophy UK is widely recognised for our positive and influential role on behalf of all patients and families affected by the conditions, working closely with leading clinicians.

I give you my very best wishes in your continuing endeavours and I hope that you have a very productive and enjoyable conference here in Newcastle.

Yours

Robert Meadowcroft
Chief Executive, Muscular Dystrophy UK

About the MRC Centre for Neuromuscular Diseases

Genetic and acquired neuromuscular diseases represent a major cause of mortality and morbidity in children and adults, affecting over 100,000 patients and their families in the UK. There remains an important gap between major science discoveries and patient benefit in these important disorders. The MRC Centre aims to continue to reduce this gap by supporting and promoting 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 Newcastle University. The Centre is building on long-established UCL-Newcastle research and clinical links. The Centre has formed reciprocal clinical and research links with other major neuromuscular research groups in Oxford and Cambridge and with 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 continues to be to translate science findings into experimental 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 £60m of grant income underpin the activities of the Centre. The Centre continues to develop 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 focused on six key areas which are obstacles to effective translation of basic science findings into patient benefit. These are: developing stratified cohorts for personalized medicine, experimental 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:

- One of our main objectives currently is to develop deeply phenotyped stratified cohorts of patients in the five disease areas we have prioritized, which are muscular dystrophies, neuromuscular channelopathies, inherited neuropathies, inclusion body myopathy and mitochondrial disease. Our biobanked samples are complimentary to these cohorts and to date we have c. 5500 patients entered into to our stratified cohorts.

- We are facilitating clinical trials in neuromuscular diseases 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, Oxford and Newcastle (NCG services). The MRC Centre is working closely with TREAT-NMD, the pan-

European network of excellence and with the NHS NIHR Biomedical research Centres. In the first phase of the MRC Centre we delivered a step change and well over 3403 patients were entered into natural history studies and clinical trials. In this phase this has now increased to 6195

- 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 have collected over 2392 human cell lines that have been invaluable for translational research including preclinical therapy evaluation including using IPS cells.

- Assessing the validity of animal models of neuromuscular disease and correlating phenotypes with human disease remains an important problem. We have linked clinical and basic scientists, thereby establishing a network and resource for elucidating the validity of mouse models.

- We have developed new outcome measures and biomarkers for NM diseases. We continue to develop new MRI techniques which have started to change the way we assess and monitor neuromuscular disease in 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 and delivered very successful four-year and three-year translational neuromuscular disease PhD programmes, and fifteen PhD students have already graduated from this programme. A cadre of 23 new students has been appointed since 2013. We prioritise the provision of exciting and inspirational translational research environments to continue to train the next generation of basic and clinical neuromuscular scientists, building future capacity in the UK.

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

Looking to the future we aim to have embedded a trial-ready culture into routine neuromuscular UK practice by 2018. We plan that every patient attending one of our core or linked centres will have the opportunity to enrol in a natural history study or clinical trial as a matter of routine. We will have developed sensitive, validated and responsive outcome measures for our 5 core diseases and expanded our national registries to include all interested UK neuromuscular centres. The next challenge will be to systematically and rationally deliver the trials needed to test the increasing number of potential therapies. To do this we are planning to develop an Experimental Therapeutic Centre in Neuromuscular Diseases which will be the natural and necessary next step to ultimately deliver successful therapies to our patients.

About Muscular Dystrophy UK

Muscular Dystrophy UK is the charity bringing individuals, families and professionals together to beat muscle-wasting conditions. We are combining skills, knowledge and resources in the UK and working with others around the world so we can improve the quality of life for people affected, and bring treatments and cures closer to reality.

Since the charity was founded in 1959, we have invested more than £55m in high quality research into the underlying molecular basis of muscle-wasting conditions. This investment has achieved positive results hence the focus of our research funding has started to shift towards the development of treatments – the bench-to-bedside transfer of promising technology. Indeed, we are delighted to have laid the foundations for the first potential treatments for Duchenne and Becker muscular dystrophy, which are now in clinical trials.

Muscular Dystrophy UK aims to accelerate the transition of promising technology into the clinic by providing support to both scientists and clinicians. We fund basic science through to pre-clinical research and, where possible, to clinical trials. We also provide logistic and financial support to create platforms where clinicians and scientists can meet, exchange experiences and discuss ideas. This event, the Eighth UK Neuromuscular Translational Research Conference here in Newcastle is one such platform.

We are also very keen to encourage early participation for UK patients in clinical trials. To ensure this, we continue to invest in the clinical trial infrastructure. We currently fund key posts of clinical trial co-ordinators and the national neuromuscular database to ensure vital patient data are available to clinicians and researchers.

With possible treatments for some conditions at last on the horizon, it is crucial that access to new drugs licensed for use in patients with neuromuscular conditions is as swift as possible. Muscular Dystrophy UK is are leading the drive for fast access to emerging treatments for UK families.

Patient Organisations

The MRC Centre and Muscular Dystrophy UK aim to develop strong links with all neuromuscular patient organisations.

UK Neuromuscular Translational Research Conference 2015
Centre for Life
Newcastle

Thursday 19th and Friday 20th March

PROGRAMME

Day 1 – Thursday 19th March

08:30 – 9:30	**Registration and Coffee**
9:30 – 9:45	**Introduction** Prof. Michael Hanna UCL Institute of Neurology
09:45 – 12:15	**Session 1: Mitochondrial Disease jointly with MBU Cambridge** Chairs: Massimo Zeviani (MBU Cambridge) and Doug Turnbull (Newcastle University)
09:45 – 10:15	**Latest developments in mitochondrial disease / mouse model** Prof. Massimo Zeviani Director, MRC Mitochondrial Biology Unit (abstract S01)
10:15 – 10:45	**Pathogenesis and Treatment of Mitochondrial Deoxynucleotide Pool Disorders** Prof. Michio Hirano Professor of Neurology, Columbia University (abstract S02)
10:45 – 11:15	**Mitochondrial muscle disease: new genes and molecular mechanisms** Prof. Rob Taylor Professor of Mitochondrial Pathology, Newcastle University (abstract S03)
11:15 – 11:45	**Coffee**
11:45 – 12:00	Platform Presentation:

The OPA1Q285STOP/RedMIT/GFP-LC3 mouse to understand the implication of mitophagy in the Autosomal Dominant Optic Atrophy
Alan Diot
University of Oxford
(abstract P34)

12:00 – 12:15 Platform Presentation:
Quantification of mitochondrial respiratory chain function in single muscle fibres: important implications for diagnosis and treatment
Mariana Rocha
Newcastle University
(abstract P35)

12:15 – 13:15 **Posters and lunch**

13:15 – 14:30 Poster guided tours

14:30 – 17:00 Session 2: Genomic data in support of translational research for neuromuscular diseases
Chairs: Francesco Muntoni (ICH, UCL) and Hanns Lochmuller (Newcastle University)

14:30– 15:00 **The role of new sequencing techniques in gene identification**
Prof. Stephan Zuchner
Interim Chair and Professor, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami
(abstract S04)

15:00 – 15:30 **Gene discovery in congenital myopathies**
Prof. Alan Beggs
Director, Manton Centre, Boston Children's Hospital
(abstract S05)

15:30 – 16:00 **Neuromics and RD-Connect – importance of data sharing in neuromuscular disorders**
Prof. Hanns Lochmuller
Chair of Experimental Myology, Newcastle University
(abstract S06)

16:00 – 16:30 **Coffee**

16:30 – 16:45 Platform presentation:
Mutations in GMPPB cause Congenital Myasthenic Syndrome and bridge myasthenic disorders with dystroglycanopathies
Katsiaryna Belaya

Nuffield Department of Clinical Neurosciences, University of Oxford
(abstract P96)

16:45 – 17:00 Platform presentation:
Correlation of the functional properties of ClC-1 variants with inheritance pattern of clinical symptoms
Roope Mannikko
UCL Institute of Neurology
(abstract P71)

17:00 – 18:00 **The Third John Walton Lecture (dedicated to the memory of David Gardner-Medwin)**
Congenital Muscular Dystrophy: The Collagen Connection
Carsten Bonnemann
Senior Investigator and Chief
Neuromuscular and Neurogenetic Disorders of Childhood Section
NIH, USA
(abstract S07)

18:00 – 19:00 **Drinks Reception and Posters**
Introduced by Robert Meadowcroft, CEO Muscular Dystrophy UK

19:00 **Aperitif followed by Gala Dinner**
Centre for Life
Newcastle

(Dress code: smart / smart casual)

Day 2 – Friday 20th March

09:00 – 11.30 Session 3: Identifying and validating new targets in NM disease
Chairs: Prof. Mary Reilly (UCL ION), Prof. Rita Horvath and Prof. Katie Bushby (Newcastle University)

09:00 – 09:30 **Endoplasmic reticulum stress in CMT neuropathy**
Prof. Lawrence Wrabetz
Director, Hunter James Kelly Research Institute; Professor of Neurology and Biochemistry; University at Buffalo
(abstract S08)

09:30 – 10:00 **Neurological presentation of altered RNA metabolism**
Prof. Rita Horvath
Professor of Neurogenetics, Newcastle University
(abstract S09)

10:00 – 10:30 **Translating Research into Drug Development- The TACT Experience**

Dr Cristina Csimma
Founding CEO Cydan Development, Inc.
(abstract S10)

10:30 – 11:00 **Coffee**

11:00 – 11:15 Platform presentation:
Hereditary Sensory Neuropathy Type 1 (HSN1) secondary to SPTLC1/2 mutations: Investigating the role of deoxysphingolipids in the pathogenesis
Dr Umaiyal Kugathasan
UCL Institute of Neurology
(abstract P80)

11:15 – 11:30 Platform presentation:
Nidogens define a novel pathway for ligand entry and signalling at the neuromuscular junction
Ione Meyer
UCL Institute of Neurology
(abstract P81)

11:30 – 12:30 **Poster flash sessions**
Chaired by Professor Michael Hanna, UCL ION

12:30 – 13:30 Lunch and Posters

13:30 – 14.45 Poster guided tours

14:45 – 15:00 Neuromuscular Breaking News
Mitochondrial Donation
Prof. Doug Turnbull, Newcastle University

15:15 – 16:45 Session 4: MRI in Neuromuscular Diseases
Chairs: Prof. Tarek Yousry (UCL ION) and Prof. Volker Straub (Newcastle University)

15:15 – 15:45 **Understanding FSHD through MRI**
Dr Giorgio Tasca
Institute of Neurology, Catholic University School of Medicine, Rome / Unit of Neuromuscular Disorders, Bambino Gesù Children's Hospital, Rome
(abstract S11)

15:45 – 16:15 **Multi-parametric MRI characterization of healthy and diseased muscle**
Prof. Bruce Damon
Vanderbilt University Institute of Imaging Science
(abstract S12)

16:15 – 16:30 Platform presentation:

Muscle MRI quantifies disease activity and severity in hypokalaemic periodic paralysis
Dr Jasper Morrow
UCL Institute of Neurology
(abstract P60)

16:30 – 16:45 Platform presentation:
Temporal Profile of T2 MRI and 1H-MRS in the MDX Mouse Model of Duchenne Muscular Dystrophy
Patrick Sweeney
Charles River Discovery Research Services, Finland
(abstract P59)

16:45 – 17:00 Poster prizes and close

Poster List

Muscular Dystrophies
Guided Poster Session Leads:
Group a (Thu) Katie Bushby and Francesco Conti
Group b (Fri) Francesco Muntoni and Volker Straub

1b	Corinne	Betts	Cardiac Implications Following Targeted Rescue of the Dystrophic Diaphragm in a Mouse Model of Duchenne Muscular Dystrophy
2b	Haiyan	Zhou	Regular systemic administration of sub-therapeutic dose of morpholino antisense oligomer PMO25 rescues SMA transgenic mice
3b	Silvère	van der Maarel	The *SMCHD1* mutation spectrum in FSHD
4b	Kiran	Shetty	Tamoxifen and Prednisolone as a combination therapy for treating Duchenne muscular dystrophy
5b	Louise	Moyle	Ret tyrosine kinase regulates satellite cell function and contributes to DUX4-mediated FSHD pathology
6b	Christopher	Banerji	InSpiRing new perspectives on Facioscapulohumeral Muscular Dystrophy
7b	Morten	Ritso	Transcriptome and Rescue Therapy Studies on mdx and C57BL/10 Cardiomyocytes Undergoing a Hypertrophic Response
8b	Golara	Torabi Farsani	Investigating novel *COL12A1* mutations in a cohort of Bethlem like patients and its underlying pathology
9b	Persefoni	Ioannou	Establishing *in vitro* and *in vivo* outcome measures in a pre-clinical treatment study for Duchenne muscular dystrophy with an inhibitor of the Na+/H+ exchanger 1
10b	Ciara	Marini Bettolo	Molecular modelling and dynamic of beta-Sarcoglycan
11b	Ursula	Moore	Heterozygous CAPN3 patients: a review of our cohort to direct further investigation
12b	Ngoc B	Lu-Nguyen	Combination antisense treatment for Duchenne muscular dystrophy: open reading frame rescue of dystrophin in conjunction with destructive exon skipping of myostatin in neonatal *mdx* mice.
13b	Elizabeth	Harris	STIM1 mutations at a common amino acid residue (p.340) identified in two individuals with a predominant muscle disease phenotype
14b	Nahla	Alshaikh	Clinical Outcomes in a large cohort of boys and adolescents with Duchenne Muscular Dystrophy.

15b	Alex	Murphy	The frequency and characterisation of cardiac involvement in female carriers of BMD or DMD: a cross sectional analysis
16b	Silvia	Dibenedetto	Polycomb Group Genes in human neuromuscular diseases.
17a	Corinne	Betts	Prevention of exercised induced cardiomyopathy following Pip-PMO treatment in dystrophic *mdx* mice
18a	Emine	Bagdatlioglu	Assessing cognitive ability in mouse models of Duchenne Muscular Dystrophy
19a	Narinder	Janghra	Biomarker development to support clinical development of utrophin modulation for Duchenne Muscular Dystrophy therapy
20a	Francesco	Catapano	MicroRNAs as biomarkers to monitor disease severity and response to antisense oligonucleotide therapy in spinal muscular atrophy
21a	Elena	Marrosu	Developing allele-specific silencing therapeutic approach using antisense oligonucleotide on Collagen 6 genes in congenital muscular dystrophy
22a	Charlotte	Morris	The National Diagnostic and Advisory Service for Limb-Girdle Muscular Dystrophies in Newcastle
23a	David	Burns	Evaluation of oral small molecules which modulate expression of utrophin and ameliorate pathology in the *mdx* mouse
24a	Rita	Barresi	Expression of truncated telethonin in a patient with limb-girdle muscular dystrophy 2G
25a	Calum	Kirk	Pathophysiology of anoctaminopathy (LGMD2L)
26a	Emma	Hoffman	Repurposed cancer therapeutics as treatments for DMD
27a	Veronica	Pini	Development of a novel approach using CRISPR/Cas9 nucleases to correct duplications in the dystrophin gene
28a	Claire	Wood	Testosterone therapy in Duchenne Muscular Dystrophy
29a	Sunil	Rodger	Care provision for adults with Duchenne muscular dystrophy in the UK: compliance with international consensus care guidelines
30a	Alison	Blain	Longitudinal characterisation of cardiac function in the *Cmah-/-mdx* mouse model of DMD
31a	Nahla	Alshaikh	Title: Vitamin D in corticosteroid treated Duchenne Muscular Dystrophy: what dose achieves serum 25OH vitamin D sufficiency?
32a	Keith	Foster	Estrogen related receptor gamma gene transfer upregulates oxidative and angiogenic factors in *mdx* mice.
33a	Julie	Dumonceaux	Nuclear protein spreading: implication for pathophysiology of neuromuscular diseases

Mitochondrial Disease

Group a (Thu) Patrick Chinnery and Massimo Zeviani

Group b (Fri) Doug Turnbull and Jo Poulton

34a	Alan	Diot	NO POSTER - SEE PLATFORM PRESENTATIONS
35a	Mariana	Rocha	NO POSTER - SEE PLATFORM PRESENTATIONS
36a	Veronika	Boczonadi	Investigation of GARS associated mitochondrial dysfunction *in vitro* and *in vivo*.
37a	Amy	Vincent	Investigating mitochondrial dysfunction in myofibrillar myopathies.
38a	Charlotte	Alston	Improving the diagnosis of paediatric mitochondrial disease using high throughput sequencing technologies
39a	Kyle	Thompson	Long-term survival in a child with severe encephalopathy, multiple respiratory chain deficiency and *GFM1* mutations
40a	Lyndsey	Craven	Reproductive Options for Women with Mitochondrial DNA Disease
41a	Jo	Poulton	Mitophagy and mitochondrial morphology in patients with the m.13051G>A mitochondrial DNA mutation
42a	Monika	Olahova	*LRPPRC* mutations cause early-onset multisystem mitochondrial disease and COX deficiency outside of the French Canadian population.
43a	Shamima	Rahman	Whole exome sequencing identifies mutations in the CLPB protein disaggregase as a novel cause of mitochondrial disease with 3-methylglutaconic aciduria
44a	Prasanth	Sivakumar	Investigation of an intronic OPA1 mutation causing optic atrophy, coupled with an intralocus modifier linked to additional extra-ocular neuromuscular disease
45a	Ewen	Sommerville	Novel alanyl-tRNA synthetase 2 (*AARS2*) mutations causing fatal infantile cardiomyopathy and respiratory failure
46a	Rebecca	Muir	A commonly used treatment for mitochondrial diabetes inhibits mitophagy in heteroplasmic patient cultures under mitochondrial energetic stress
47a	Julia	Maddison	Reproductive Decision Making in Mitochondrial Patients: A Qualitative Investigation of Women's Experience.
48b	Emily	McILwaine	PRDM9 has zinc fingers in many pies
49b	Cecilia	Jimenez Mallebrera	Growth and Differentiation Factor-15 and Fibroblast Growth Factor-21 are comparably sensitive and specific biomarkers of mitochondrial diseases

50b	Marina	Bartsakoulia	Studying the effect of L-cysteine in mitochondrial diseases
51b	Hannah	Rosa	Why do patients with mitochondrial disease get worse over time: An investigation into genetic and cellular mechanisms of disease progression
52b	Jane	Newman	Gait analysis in patients with mitochondrial disease
53b	Jane	Newman	Gait analysis: Accuracy as a clinician reported measure in mitochondrial disease
54b	Yi	Ng	Cardiac involvement in adult mitochondrial disease: The need for a genotype-specific management guideline
55b	Conrad	Smith	A case of hepatocerebral mitochondrial DNA depletion syndrome caused by two novel splicing mutations in the *DGUOK* gene
56b	Carl	Fratter	Whole human mitochondrial genome next generation sequencing
57b	Louise	King	Mitophagy deficiencies in mitochondrial DNA disease
58b	Patrick	Yu Wai Man	Chronic progressive external ophthalmoplegia – molecular genetic features and neurological burden

MRI

Guided Poster Session Leads: John Thornton and Andy Blamire

59	Patrick	Sweeney	NO POSTER - SEE PLATFORM PRESENTATIONS
60	Jasper	Morrow	NO POSTER - SEE PLATFORM PRESENTATIONS
61	Kieren	Hollingsworth	Abnormal resting phosphorus metabolism in skeletal muscle in limb girdle muscular dystrophy 2I (LGMD2I)
62	Courtney	Bishop	Magnetic Resonance Imaging Assessments of two doses of Drisapersen in the Treatment of Ambulant Boys with Duchenne Muscular Dystrophy
63	Enrico	Bugiardini	Utility of muscle MRI in distal myopathies
64	Fiona	Smith	Quantitative imaging and spectroscopy to assess clinical outcome in a natural history study of Dysferlinopathy
65	Matthew	Evans	Accurate slice selection improves responsiveness of quantitative lower limb muscle MRI in CMT1A patients
66	Emine	Bagdatlioglu	Investigating the effect of dystrophin deficiency on brain function in mouse models of Duchenne Muscular Dystrophy
67	Claire	Wood	MYO-MRI: The creation of 'Scan Bank'
68	Paola	Porcari	Diffusion MR study at 7T of hindlimb muscles in dystrophic mice

69	Elizabeth	Greally	Manganese enhanced muscle MRI as a Sensitive Outcome Measure of Dystrophin Restoration in the mdx Mouse
70	Chris	Sinclair	MRI Quantitation of Muscle Water with IDEAL-CPMG

Muscle Channelopathies and Myasthenia Gravis
Guided Poster Session Leads: Mike Hanna and Thomas Voit

71	Roope	Mannikko	NO POSTER - SEE PLATFORM PRESENTATIONS
72	Michael	Thor	*SCN4A* mutations in a patient with congenital myopathy
73	Neta	Amior	A novel drug model of Hypokalaemic Periodic Paralysis in neonatal cell cultures
74	Enrico	Bugiardini	A new sodium channel myotonia (SCM) mutation in the Nav1.4 DII-S4S5 linker
75	Emanuela	Agazzi	Rituximab treatment in two subacute resistant bulbar myasthenia cases
76	Lauren	Phillips	Exome sequencing in Congenital Myasthenic Syndromes
77	Karen	Suetterlin	An Investigation into the Side Effect Profile, Safety Profile and Perceived Efficacy of Mexiletine for the Treatment of Myotonia in a Cohort of Patients with Dystrophic and Non-dystrophic Myotonia
78	Victoria	Selby	Developing validated specific home based assessment tools for monitoring fluctuations in fatigability and muscle performance in adult and paediatric myasthenia patients
79	Francesco	Conti	Integrins are required for synaptic transmission and development of the neuromuscular junction

Peripheral Nerve Disease
Guided Poster Session Leads: Mary Reilly and Stephan Zuchner

80	Maiya	Kugathasan	NO POSTER - SEE PLATFORM PRESENTATIONS
81	Ione	Meyer	NO POSTER - SEE PLATFORM PRESENTATIONS
82	Pedro	Tomaselli	Clinical and genetic spectrum of X-linked Charcot-Marie-Tooth disease due to mutations in the 5' untranslated region of *GJB1*.
83	Boglarka	Bansagi	Aminoacyl-tRNA synthetases (ARS) related inherited axonal neuropathies in the North England cohort of CMT patients
84	Emanuela	Agazzi	CIDP in overlap syndrome autoimmune hepatitis
85	Mariola	Skorupinska	Survey of pregnancy in patients with Charcot-Marie-Tooth

			disease and related hereditary neuropathies
86	Matilde	Laura	Management of the Orthopaedic Complications in Charcot Marie Tooth Disease Patients
87	Matilde	Laura	Survey of Current Management of Orthopaedic Complications in Charcot Marie Tooth Disease Patients
88	Tayyibah	Ali	Charcot-Marie-Tooth and Centronuclear myopathy induced mechanistic impairment in endocytosis
89	Trupti	Bhandari	Charcot Marie Tooth disease Natural History Study: evaluation of the CMT Paediatric Scale in the UK cohort
90	Andreea	Manole	Riboflavin Transporter Neuronopathy
91	Daniyal	Daud	Whole exome sequencing – a Pandora's box?
92	Emma	Wilson	Cellular pathomechanisms of Hereditary Sensory and Autonomic Neuropathy type 1 (HSAN-1) in primary motor neurons
93	Alex	Rossor	Plasma Neurofilament Heavy Chain Levels in Charcot-Marie-Tooth Disease
94	Verna	Sarajarvi	Investigating CMT-2 pathology in patient fibroblasts: a morphological and functional study.
95	Gita	Ramdharry	Exploring the causes of falls and balance impairments in people with NEUROPATHY

<u>Glycosylation Disorders, IBM, Muscle Satellite cells and IPS Cells</u>
Guided Poster Session Leads: Hanns Lochmuller and Linda Greensmith

96	Katsiaryna	Belaya	NO POSTER - SEE PLATFORM PRESENTATIONS
97	Renata	Scalco	A phase II pilot study to explore treatment with Sodium valproate in Adults with McArdle Disease (Glycogen Storage Disorder Type V, GSDV)
98	Sue	Brown	FKRP is not required for glycosylation of α- dystroglycan in the heart during development
99	Jihee	Kim	Dystroglycan glycosylation and secondary myogenesis in FKRP Deficient ($FKRP^{KD}$) Mice.
100	Helen	Booler	Cajal-Retzius cell mislocalisation correlates with the severity of structural brain defects in mouse models of dystroglycanopathy.
101	Qiang	Gang	Using whole-exome sequencing to identify mutations of *SQSTM1* and *VCP* in inclusion body myositis
102	Charlotte	Spicer	Investigating the effects of pharmacological up-regulation of

			the heat shock response in a transgenic mouse model of inclusion body myopathy
103	Katarzyna	Swist-Szulik	Does NRLP3 inflammasome activation take part in muscle inflammation?
104	Teresinha	Evangelista	Mutational spectrum and phenotypic variability of VCP related neurological disease in the UK
105	Giulia	Ferrari	Towards a genomic integration-free, iPS cell and human artificial chromosome-based therapy for Duchenne muscular dystrophy
106	Beatrice	Lana	Overcoming challenges in muscular dystrophy research: Genome editing tools for targeted gene correction in patient-specific iPSC.
107	Nicolas	Figeac	Developing 3D scaffolds to support Myogenesis

Databases, Diagnostics and Clinical Practice

Guided Poster Session Leads: Henry Houlden and Rita Barresi

108	Hannah	Steele	Cardiac disease is under recognised in patients with HMERF
109	Danielle	Ramsey	SMA REACH UK One Year On: The Evolution of Robust Functional Outcome Measures for Spinal Muscular Atrophy Type 2 & 3
110	Libby	Wood	UK Patient Registry for Facioscapulohumeral Muscular Dystrophy (FSHD)
111	Oksana	Pogoryelova	GNE Myopathy disease monitoring program as a tool in translation research for ultra-rare disease.
112	Tania	Smertenko	Exome sequencing in undiagnosed inherited and sporadic ataxias
113	Rebecca	Whittington	Gene panel testing reveals that a high proportion of patients with inherited peripheral neuropathy have autosomal recessive disease.
114	Ana	Topf	Next Generation Sequencing in Neuromuscular Disease: the Newcastle MRC Muscle Centre Effort
115	Mojgan	Reza	MRC Centre for Neuromuscular Diseases Biobank

Other

Guided Poster Session Leads: Rob Taylor and Pedro Machado

116	Michele	Giunta	Zebrafish as a model system in RNA metabolism deficiencies.

117	Bernadett	Kalmar	Introducing a specialized Masters Course in Neuromuscular Diseases: Educating the next generation of scientists, clinicians and Professionals allied to medicine at UCL Institute of Neurology.
118	Johanna	Prueller	Static mechanical stimulation improves myogenic differentiation in a novel bioreactor-based 3D culture model
119	Giuseppa	Piras	Red blood cells as therapeutic carrier in monogenic disorders
120	J M	de Winter	Investigating the cause of muscle weakness in thin filament myopathies
121	Marta	Bertoli	Joints contractures, ptosis and external ophthalmoplegia caused by a novel autosomal dominant mutation in *PIEZO2*.
122	Daniel	Cox	Modelling a novel *INPP5K* mutation associated with Marinesco-Sjögren Syndrome in zebrafish
123	Cecilia	Jimenez Moreno	OPTIMISTIC*:* Observational Prolonged Trial In Myotonic dystrophy type 1 to *I*mprove Quality of Life- Standards, a Target Identification Collaboration.
124	Emily	O' Connor	Identification of causal genes in Congenital Myasthenic Syndrome by whole exome sequencing.

Abstracts: Invited Speakers

Thursday 19th March 2015

S01

Identification and characterization of new mitochondrial disease genes

Massimo Zeviani[1], [2], Aurelio Reyes[1], Carlo Viscomi[1], Gabriele Civiletto[1], Raffaele Cerutti[1], Erika Fernandez-Vizarra[1], Dario Brunetti[1], Emanuela Bottani[1].

1 MRC Mitochondrial Biology Unit

[2] University of Cambridge, UK

Mitochondria are the major source of ATP that is synthesized by the respiratory chain through the process of oxidative phosphorylation (OXPHOS), a complex biochemical process carried out through the dual control of physically separated, but functionally interrelated, genomes, nuclear and mitochondrial DNAs. The genetic and biochemical intricacy of mitochondrial bioenergetics explains the extreme heterogeneity of mitochondrial disorders, a group of highly invalidating human conditions, for which no effective treatment is nowadays available. In addition to bioenergetic failure, other mechanisms are probably predominant in the pathogenesis of specific syndromes, such as alterations of cellular redox status, the production of reactive oxygen species, compromised Ca^{2+} homeostasis, mitochondrial protein and organelle quality control, and mitochondrial pathways of apoptosis. By investigating selected families and patients, we have identified several new disease genes, each responsible of distinct defects of the respiratory chain, mtDNA metabolism, or both. Recently published and still unpublished findings will be presented and discussed. Structural analysis and the creation of ad hoc recombinant lines in yeast, flies, and mice have allowed us to dissect out the molecular consequences of the ablation or defects of some of these proteins, and their physical status in normal and disease conditions. These models have also been exploited to implement experimental therapeutic strategies, based on gene and cell replacement, or pharmacological control of mitochondrial biogenesis.

S02

Pathogenesis and treatment of mitochondrial deoxynucleotide pool disorders

Michio Hirano[1], Caterina Garone[1], Beatriz Garcia-Diaz[1], Catarina Quinzii[1], Jörg P. Halter [2], Michael Schüpbach[3], John LP Thompson[1], and Ramon Martí[4].

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[2] University Hospital Basel, Switzerland

[3] University of Bern, Switzerland

[4]University Hospital Vall d'Hebron, Barcelona, Spain

Maintenance of mitochondrial DNA (mtDNA) requires a balanced pool of deoxynucleotide triphosphate (dNTP) precursors. Defects of deoxynucleoside/deoxynucleotide metabolism cause mtDNA instability (depletion, multiple deletions, and point mutation) that cause severe disorders such as mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and hepatocerebral or myopathic forms of mtDNA depletion syndrome. MNGIE is an autosomal recessive disease due to *TYMP* mutations that disrupt cytosolic thymidine phosphorylase (TP) activity. TP deficiency causes systemic toxic accumulations of thymidine and deoxyuridine nucleosides that, in turn, cause mitochondrial dNTP pool imbalance. Our studies of TP deficient mice have confirmed the pathogenic mechanism and support the notion that restoration of TP activity via hematopoetic stem cell transplantation (HSCT) or gene therapy will be effective. HSCT in 24 MNGIE patients has demonstrated efficacy in 9 cases with successful transplants but morbidity and mortality were unacceptably high. A consensus protocol for HSCT in MNGIE is being assessed in a Phase I safety study. In contrast to the accumulation of toxic metabolites in MNGIE,

thymidine kinase 2 (TK2) deficiency causes a lack of mitochondrial dNTPs that typically causes severe depletion of mtDNA and myopathy in early childhood. Our Tk2 knockin mouse model develops severe mtDNA depletion that is fatal. Administration of deoxynucleotide monophosphates to bypass the biochemical defect in our Tk2 knockin mice ameliorated the mtDNA depletion and extended the lifespan of the mutant animals by 2-3 fold. Thus, our translational studies of disorders of mitochondrial deoxynucleotide pools have provided pathomechanistic insights and promising therapeutic approaches.

S03

Mitochondrial muscle disease: new genes and molecular mechanisms

Robert W. Taylor
Wellcome Trust Centre for Mitochondrial Research and UK NHS Highly Specialised Mitochondrial Service, Newcastle University, UK

Mitochondrial diseases are an important cause of human morbidity and mortality across the age spectrum and currently lack curative treatments. These debilitating and often fatal diseases are caused by mutations in mitochondrial-encoded (37 genes) or nuclear-encoded (>1400 genes) proteins that impair the efficiency of oxidative phosphorylation (OXPHOS) responsible for generating ATP, the energy currency of the cell. The clinical and genetic heterogeneity exhibited by mitochondrial disorders and the complexities of mitochondrial genetics makes their investigation and diagnosis a considerable challenge, necessitating a multidisciplinary laboratory approach incorporating the biochemical assessment of OXPHOS function to guide molecular genetic screening toward a definitive diagnosis. Our research aims to delineate the molecular mechanisms underlying primary mitochondrial dysfunction through a programme of gene discovery aligned to patient cohorts identified through our national clinical-diagnostic service, translating this information into the development of rapid, diagnostic tests which can inform and influence the reproductive options available to families, with several examples being presented within this talk.

S04

The role of new sequencing techniques in gene identification

Stephan Zuchner
Department of Human Genetics and Hussman Institute for Human Genomics, University of Miami Miller School of Medicine

Next-generation sequencing has significantly increased the pace of gene discovery in neuromuscular diseases. Further, it increasingly allows for the delineation and precise description of phenotypic spectra of disease genes, the resulting phenotypic overlap between clinical entities, and in some areas this is challenging the existing classification of disorders. With the decreasing price for genome sequencing, we will witness an even larger expansion of genetic data in the coming years. Going forward it appears inevitable that intelligent and safe sharing of data on genetic variation is a precondition to overcome the challenges of determining pathogenicity and to allow for the unbiased determination of validity of new disease genes. Local and global efforts are now taking shape to address this pressing issue, which will eventually lead to a global understanding of genetic variation in our species, and hence, improved and standardized diagnostic approaches in neuromuscular disease. This presentation will review these issues and highlight work done in the Zuchner lab, the Inherited Neuropathy Consortium, and recent efforts of the Global Alliance for Genomics and Health (GA4GH).

S05

Gene discovery in congenital myopathies

Alan H. Beggs
Division of Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, Boston, U.S.A

In recent years, the concept and diagnostic criteria for congenital myopathies have evolved from a clinicopathological definition, reliant on documentation of specific histopathological abnormalities in skeletal muscle, to one increasingly based on identification of the underlying genetic basis for the condition. Genetic characterization of these diseases has demonstrated a high degree of genetic heterogeneity, as well as significant phenotypic overlap, among and between a variety of congenital myopathies including core myopathies, rod myopathies, centronuclear myopathies and more. Initially, gene identifications relied on linkage mapping and positional cloning utilizing large families with multiple affected individuals. As the biological functions for these early genes became appreciated, the field increasingly relied on a candidate gene approach for new disease gene identification. Most recently, the development of next generation sequencing has allowed for a return to unbiased methods that have led to discoveries of new genes involved with pathways hitherto unrecognized as contributing to the pathophysiology of congenital myopathies. Congenital myopathies are now recognized as a clinically, pathologically, and genetically heterogeneous collection of conditions with a shared set of underlying pathophysiological mechanisms largely involving defective excitation-contraction coupling and contractile machinery.

S06

NeurOmics and RD-Connect – importance of data sharing in neuromuscular disorders

Hanns Lochmüller

The John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK

The very nature of their low frequency means that research into rare neuromuscular diseases, including neuromuscular disorders, can be particularly challenging. International collaboration is essential to avoid duplication of effort, missed opportunities and unnecessary multiplication of unlinked, siloed datasets. The development of novel omics technologies in recent years has offered new methods to identify novel disease and modifier genes, elucidate disease mechanisms, discover biomarkers, explain the complex interplay between the genome and the environment and ultimately identify novel therapeutic targets. To date, however, concrete benefits to patients from these personalised and stratified medicine approaches have been limited by the lack of data sharing and lack of interoperability between individual efforts.

Several initiatives have attempted to address these challenges and promote open collaboration to maximise interoperability of data and reuse of results. RD-Connect (http://rd-connect.eu/) and NeurOmics (http://rd-neuromics.eu/) are two flagship IRDiRC (http://www.irdirc.org/) projects funded by the European Union from 2012 onwards that provide a proof-of-concept for harmonisation and data sharing. RD-Connect is an infrastructure project that is developing data sharing mechanisms and omics analysis and bioinformatics tools that are incorporated into an integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research. NeurOmics, an omic project which focuses on rare neuromuscular and neurodegenerative disorders, will feed the data it generates into the RD-Connect platform.

Within the NeurOmics consortium, whole exome sequencing of ~ 500 unsolved cases has to date identified > 50 novel genes associated with nine different rare neuromuscular and neurodegenerative diseases, with many more expected. This translates directly into patient benefit, with many families receiving a diagnosis for the first time. The discovery of these new genes is also used to update diagnostic panels, inform other diagnostic tests, and to check for the same mutations in genomic data from other research datasets.

S07

Congenital Muscular Dystrophy: The Collagen Connection

Carsten G. Bönnemann[1].

[1]National Institute of Neurological Disorders and Stroke, NIH, Bethesda, USA

Congenital muscular dystrophies (CMDs) are dystrophic myopathies with clinical manifestations typically evident at birth, but with a wide spectrum of severities and age ranges at presentation. The CMDs are genetically quite heterogeneous, however, the three most common forms of congenital muscular dystrophy are all affecting components to the muscle extracellular matrix and its receptors on muscle. These three forms are the alpha-dystroglycanopathies, LAMA2-related (merosin deficient) CMD and the spectrum of COL6-related muscular dystrophies, which are arguably the most matrix-based of the three. In this talk I will provide an overview outlining progress in the clinical characterization, natural history and genetic basis of these conditions and provide data pertinent to translational approaches to the collagen VI related conditions, including "knockdown" approaches to the prevalent dominantly acting COL6 mutations. Furthermore, I will introduce collagen XII related disease as a newer member of the muscle collagen disorders.

Friday 20th March 2015

S08

Endoplasmic reticulum stress in CMT neuropathy

Maria Paola Sidoli[1], Nicolo Müsner[1], Maurizio D'Antonio[2], Maria Laura Feltri[1], Lawrence Wrabetz[1]
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Endoplasmic Reticulum (ER) Stress has been implicated in the pathogenesis of both acquired and hereditary neuropathies. For example, mutant proteins associated with several Charcot-Marie-Tooth (CMT) neuropathies provoke ER stress and an unfolded protein response (UPR), and perturbations of UPR alter the severity of neuropathy. P0 glycoprotein is abundantly synthesized in myelinating Schwann cells. The mutant P0S63del causes CMT1B neuropathy in humans, and a very similar demyelinating neuropathy in S63del transgenic mice. P0S63del is retained in the ER of Schwann cells where it promotes unfolded protein stress and elicits an UPR associated with translational attenuation. We have shown that ablation of CHOP, a UPR mediator downstream of the PERK kinase sensor of unfolded proteins, ameliorates demyelination in S63del nerves. In addition, Gadd34 is a detrimental effector of CHOP that reactivates translation too aggressively in myelinating Schwann cells. Limitation of Gadd34 function improves myelination in S63del nerves, and reduces accumulation of P0S63del in the ER by maintaining translational attenuation. Surprisingly, ablation of PERK in Schwann cells, which activates translation and therefore should worsen demyelination, actually improves myelination and neuropathy. PERK may have additional targets outside of the UPR. Limiting Gadd34 in order to reset translational homeostasis may provide a therapeutic strategy in tissues impaired by misfolded proteins.

S09

Deficient RNA metabolism as a novel target in neuromuscular disease

Professor Rita Horvath
The John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases, Institute of Genetic Medicine, Newcastle University

The importance of RNA processing in neurodegeneration is highlighted with a rapidly increasing number of human neurogenetic diseases caused by mutations in proteins involved in mRNA metabolism including spinal muscular atrophy (SMA) and pontocerebellar hypoplasias (PCH). A novel mechanism of RNA-associated neurodegeneration has been suggested by the identification of mutations in genes encoding human exosome components. The exosome is a multi-protein complex, required for degradation of AU-rich element (ARE)

containing mRNAs, which is an important regulatory step in gene expression. However the human exosome may also regulate gene expression via diverse RNA processing reactions.
Mutations in *EXOSC3* were reported in pontocerebellar hypoplasia and spinal motor neuron abnormalities (PCH1), and our group recently identified mutations in a novel gene *EXOSC8*, encoding a core component of the human exosome in children with overlapping symptoms of cerebellar hypoplasia, spinal muscular atrophy and hypomyelination. In a single patient with spinal muscular atrophy (SMA) a potentially disease-causing mutation was detected in *RBM7*, a co-factor of the exosome complex which binds non-coding PROMoter uPstream Transcripts (PROMPT)s. Despite the complex and central role of the exosome in RNA metabolism in all cell types it is so far not known why and how only specific neuronal cells are affected by exosomal protein deficiency.
We investigated how abnormal RNA metabolism due to defect of exosomal proteins affect gene expression in different human cells *in vitro* and in parallel we studied zebrafish models of exosomal protein deficiencies. Identifying the biochemical pathways of RNA metabolism alterations due to different types of exosome dysfunction may explain why mutations in separate components of the exosome cause different disease presentations, and may identify potential pathways which can be targeted to develop experimental therapies.

S10

Translating research into drug development – the TACT experience

Dr Cristina Csimma
Csimma LLC
Founding CEO Cydan Development, Inc.

The last few years have shown an unprecedented level of activity in orphan drug development and in investments in rare disease companies. With the exponential growth of programs with clinical translational goals and a limited number of patients, the need for effective mechanisms and partnerships to facilitate well informed decisions to support advancing programs to the clinic is greater than ever.
The TREAT-NMD Advisory Committee for Therapeutics (TACT) TACT was established to provide the neuromuscular community with a unique resource of multidisciplinary drug development and disease expertise. It includes representatives from academia, industry, patient foundations and government research bodies that provide independent and objective guidance, unrelated to a funding stream, to more effectively advance orphan drug development. The confidential, comprehensive reviews and resulting recommendations emphasize a rigorous, milestone-driven approach enabling optimal use of funding and resources and resulting in programs that are much more likely to be funded or partnered. Since its formation in 2009, the TACT has reviewed 30 programs in several neuromuscular rare diseases with large unmet need. In addition to the direct contribution to the programs reviewed, TACT also provides significant educational value to applicants, such as a much deeper appreciation of the drug development requirements by academic researchers, and a greater understanding of the specific rare diseases and associated patient communities by industry developers. The mutual understanding of the complex drug development process helps bridge the historical communication gap between key stake holders. Based on its impact to date, the TACT model is being evaluated for adoption by other rare diseases outside of the neuromuscular field.

S11

Understanding FSHD through MRI

Giorgio Tasca
Institute of Neurology, Catholic University School of Medicine, Rome / Unit of Neuromuscular Disorders, Bambino Gesù Children's Hospital, Rome

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most frequent muscle diseases, characterized by a unique, non-conventional genetic mechanism. Great efforts have been put in the clarification of the molecular etiology of FSHD, but the pathophysiology downstream the genetic lesion is still largely elusive.

Using MRI to characterize muscle involvement and follow patients up, a picture has emerged in which sequential bursts of degeneration involve individual muscles in an asynchronous manner. This peculiar radiological progression is in line with results obtained with multidisciplinary approaches, thus configuring FSHD as a "muscle by muscle" disease.
In this context, a common feature of FSHD is the presence of areas of hypersignal on STIR (short-tau inversion recovery) sequences, which represent areas of muscle edema/inflammation. Imaging and molecular evidences have accumulated pointing toward the fact that these STIR+ lesions mark a different phase of disease at single muscle level. Consequently, even though the exact role of inflammation is not fully understood, the detection of these abnormalities is surely important to monitor disease evolution.
Even in the frame of this highly peculiar disease progression, which contributes to the pronounced clinical variability and atypical presentations, still muscle imaging is able to identify muscles that are more likely to be involved, and others that are selectively spared, as it happens in many genetic muscle disorders. Reasons of this different susceptibility are still unknown and definitely represent an intriguing field of research.

S12

Multiparametric MRI characterization of inflammatory muscle diseases

Bruce Damon
Institute of Imaging Science and Departments of Radiology and Radiological Sciences, Biomedical Engineering, and Molecular Physiology and Biophysics, Vanderbilt University, Nashville TN USA

The idiopathic inflammatory myopathies, such as polymyositis (PM) and dermatomyositis (DM), are characterized by muscle atrophy with or without fat replacement and inflammation. These chronic diseases may fluctuate in severity over time, requiring periodic adjustment of medication regimes. However, typically used quantitative biomarkers, such as serum CK levels, are not always well correlated with clinical status; nor are they sensitive to the degree or location of muscle involvement. The overarching goal of these studies is to develop quantitative MRI methods as biomarkers for muscle disease in PM and DM. Studies performed in murine models of muscle inflammation reveal that the longitudinal and transverse relaxation time constants (T_1 and T_2, respectively), the ratio of macromolecular-to-free water protons (the pool size ratio, PSR), the self-diffusion coefficient for free water, and the washout rate of an MRI contrast agent are sensitive to inflammation. Studies in human PM and DM patients revealed T_1 and T_2 elevations, increased intramuscular fat content, a higher self-diffusion coefficient for water, and muscle structural abnormalities in these patients. These studies support the use of these method to characterize muscle disease progression in PM and DM.

Abstracts: Posters and Platform Presentations

‡indicates a platform or flash presentation

Muscular Dystrophies

‡P01

Cardiac implications following targeted rescue of the dystrophic diaphragm in a mouse model of Duchenne muscular dystrophy

Corinne A. Betts[1], Amer F. Saleh[2], Carolyn A. Carr[1], Suzan M. Hammond[1], Caroline Godfrey[1], Graham McClorey[1], Caroline Woffindale[1], Kieran Clarke[1], Michael J. Gait[2], Matthew J. A. Wood[1,*]

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Duchenne muscular dystrophy (DMD) is caused by absence of the integral structural protein, dystrophin, which renders muscle fibres susceptible to injury and degeneration. This ultimately results in cardiorespiratory dysfunction, which is the predominant cause of death in DMD patients, and highlights the importance of therapeutic targeting of the cardiorespiratory system. There is evidence to suggest that restoring dystrophin in the diaphragm improves both respiratory and cardiac function, however these studies do not adequately show the contribution of the diaphragm alone. Here we specifically examined restoration of dystrophin in the diaphragm and assessed cardiac function by MRI. This approach reduced diaphragmatic pathology but did not improve cardiac function or pathology, with or without exercise. Interestingly, exercise resulted in a reduction of dystrophin protein and exon skipping in the diaphragm. This suggests that treatment regimens may require modification in more active patients. In conclusion, whilst the diaphragm is an important respiratory muscle, it is likely that dystrophin needs to be restored in other tissues, including accessory respiratory muscles, and of course the heart itself for appropriate therapeutic outcomes. This supports the requirement of a body-wide therapy to treat this debilitating disease.

‡P02

Regular systemic administration of sub-therapeutic dose of morpholino antisense oligomer PMO25 rescues SMA transgenic mice

Haiyan Zhou, Jinhong Meng, Elena Marrosu, Jennifer Morgan and Francesco Muntoni

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Background: Antisense oligonucleotides (AOs) therapy has recently demonstrated its promising efficacy in preclinical models of spinal muscular atrophy (SMA). We and others have reported the dramatic rescue of severe SMA transgenic mice by a single administration of AOs at high dose in neonatal mice.

Aims: We aim to: (1) generate a mouse model of intermediate severity between the most severe type I and the mild type III by low-dose morpholino antisense oligomer (PMO) in type I SMA mice; (2) explore the therapeutic window in the less severe model; (3) investigate the efficacy of regular repeated systemic administration of low-dose PMO in the treatment of SMA.

Methods and Materials: Severe SMA mice were given a single low-dose of a 25-mer PMO, PMO25, which we have previously demonstrated to induce *SMN2* exon 7 inclusion, at postnatal day 0 (PND0). Subsequent systemic injections were performed thereafter at different time points. The investigated parameters include survival, splicing of *hSMN2* and neuromuscular system pathologies.

Results: The SMA mice treated with a single low-dose administration of PMO25 exhibit a modestly extended lifespan (from 10 to 20 days) with moderately improved neuromuscular histopathology. Mice receiving a second low-dose systemic administration on PND5 significantly increased the survival of single low-dose pre-treated SMA mice from 20 days to over 80 days. The efficacy diminished when the second low-dose injection was delayed to PND10. Repeated low-dose systemic administration at 2-weeks intervals prevented the occurrence of ear and tail necrosis to over 100 days during the period of injections.
Conclusion: There is a clear early requirement for SMN protein in SMA. However, in some conditions that if the level of SMN protein replacement is not optimal, subsequent pulse of AON and presumably higher levels of SMN protein at a later stage can still have a role in improving survival and phenotypes. In addition, restoration of SMN protein in peripheral tissues by systemic administration during the later stage may still provide therapeutic benefit.

‡P03

The *SMCHD1* mutation spectrum in FSHD

Richard JLF Lemmers[1], Marlinde L van den Boogaard[1], Patrick J van der Vliet[1], Judit Balog[1], Bert Bakker[2], Stephen J Tapscott[3], Sabrina Sacconi[4], Rabi Tawil[5] and **Silvère M van der Maarel**[1]
1 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
2 Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands
3 Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, USA
4 Centre de référence des Maladies neuromusculaires, Nice University Hospital, Nice, France
5 Department of Neurology, University of Rochester Medical Center, Rochester , USA

Facioscapulohumeral muscular dystrophy (FSHD) is a common myopathy that progressively affects the facial and upper extremity muscles with marked clinical variability. The most common form, FSHD1, is caused by a D4Z4 repeat array contraction to a size of 1-10 units (normal range 10-100 units). The less common form, FSHD2, is most often caused by loss of function mutations in the structural maintenance of chromosomes flexible hinge domain containing 1 (*SMCHD1*) gene on chromosome 18p in combination with a medium short D4Z4 repeat array (11-16 units). SMCHD1 is a chromatin modifier involved in the maintenance of D4Z4 methylation and a repressed chromatin structure. Both FSHD situations lead to a partial failure in repeat mediated epigenetic repression of the D4Z4 repeat array and transcriptional derepression of D4Z4-encoded DUX4 protein that is toxic to muscle cells.
We established the *SMCHD1* mutation spectrum in a large cohort of FSHD2 individuals and demonstrate that the variability in clinical severity is, at least in part, correlating with individual differences in the degree of CpG hypomethylation at D4Z4. The mutation spectrum ranges from large deletions causing *SMCHD1* hemizygosity, to missense and nonsense mutations, as well as in- and out of frame splice site and small indel mutations. In some FSHD1 families, *SMCHD1* can also act as a modifier gene and in addition to the autosomal dominant inheritance, we have also identified a family with semidominant inheritance.
This mutation spectrum shows that the epigenetic susceptibility to somatic DUX4 expression depends on the D4Z4 repeat array size and the nature of the *SMCHD1* mutation with SMCHD1 open reading frame-preserving mutations being more deleterious for the maintenance of a repressive D4Z4 chromatin state than open reading frame-disrupting mutations. The involvement of SMCHD1 in FSHD2 and FSHD1 pathogenesis, positions SMCHD1 central the disease mechanisms and warrants further studies into SMCHD1 as therapeutic target.

‡P04

Tamoxifen and Prednisolone as a combination therapy for treating Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting, recessive X-linked disorder which affects ~1 in 3500 males, and arises due to abnormalities in the dystrophin gene. The 'gold standard' of care is the use of Prednisolone (PRED), a corticosteroid which decreases inflammation in the muscle, but there is a pressing need for new therapies. Dystrophin plays a vital role in preventing contraction-induced damage to the sarcolemma caused by the mechanical stresses imposed on it. DMD patients have little to no dystrophin; the muscle pathology shows extensive fibrosis, and the muscle area is substituted with connective tissue and fat cells. Recent studies administering Tamoxifen (TAM) in the mdx mouse (an animal model for DMD) showed decreased fibrosis in the diaphragm and improved dystrophic muscle structure and function. The aim of this study was to test the hypothesis that TAM and PRED as a combination therapy decreases fibrosis and improves muscle strength in the mdx mouse. Briefly, 12-week old male mdx mice were separated into four treatment groups: TAM, PRED, TAM/PRED and mdx- Control (mdx-CON); age-matched C57/BL10ScSn male mice were kept as wild-type controls (BL10-CON) and the mice were treated for 12 weeks with the drugs being incorporated in their food. At the end of treatment, the mice underwent a muscle physiology protocol to assess muscle strength and protection against eccentric contraction induced damage, with further post-mortem muscle pathology analysis. The results show the combination therapy made the tibialis anterior muscle stronger, it offered some protection against eccentric contraction induced damage (albeit not as much as PRED), it also decreased fibrosis and the amount of macrophages in the diaphragm. In conclusion, the combination therapy of TAM/PRED has shown promising results, and could be used as a potential therapy for DMD.

P05

Ret tyrosine kinase regulates satellite cell function and contributes to DUX4-mediated FSHD pathology

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant inherited myopathy, resulting in sporadic expression of the double homeodomain protein DUX4 in adult muscle. Over-expression of DUX4 inhibits myogenic differentiation and is pro-apoptotic in cultured myoblasts, suggesting that DUX4 expression in man suppresses adult myogenesis.

Aims: To identify key DUX4 target genes that contribute to the pathogenic phenotype and determine whether modulation of these genes can rescue pathology.

Methods: A microarray was performed on murine satellite cell-derived myoblasts expressing DUX4, followed by a series of overexpression, knock-down and rescue experiments, to determine the endogenous and pathogenic roles of DUX4 target genes.

Results: Expression of the receptor tyrosine kinase *Ret* was significantly upregulated by DUX4-expressing myoblasts, suggesting a potential role in FSHD pathology. *Ret* was dynamically expressed during satellite cell differentiation. Constitutive expression of either *Ret9* or *Ret51* isoforms increased myoblast proliferation but did not affect myotube formation or expression of the myogenic regulatory factors *MyoD*, *Myf5* and *Myogenin*. siRNA-mediated knockdown of *Ret* reduced proliferation and *Pax7* expression, but increased expression of *Myogenin*: suggesting that *Ret* keeps myoblasts in a proliferative state.

To test if *Ret* is important in DUX4-mediated pathology, we used siRNA against *Ret* in DUX4-expressing myoblasts and found that we were able to rescue myogenic differentiation. We further validated the critical role of *Ret* as a DUX4 target using a clinically approved drug that inhibits RET function

Conclusion: RET signalling maintains satellite cells in a proliferative state. DUX4-mediated activation of RET prevents myogenic differentiation and could contribute to FSHD pathology by preventing satellite cells from repairing muscle. Our rescue of aspects of DUX4-induced muscle pathology by a small molecule inhibitor highlights the potential for this pathway as a drug target for treating FSHD.

P06

InSpiRing new perspectives on facioscapulohumeral muscular dystrophy

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is characterised by selective skeletal muscle atrophy and is currently incurable. Genetically, FSHD is associated with contraction or hypomethylation of *D4Z4* repeats on chromosome 4. This causes aberrant expression of the transcription factor *DUX4* from an open reading frame in the last *D4Z4* unit. While many genes have been implicated in FSHD pathophysiology, an integrated molecular model is lacking.

Aims: Our objective was to use novel mathematical tools to generate a molecular network describing protein interactions perturbed in FSHD, via meta-analysis of FSHD patient muscle gene expression data.

Methods: We developed a differential network methodology, Interactome Sparsification and Rewiring (*InSpiRe*), which detects network rewiring between phenotypes by integrating gene expression data with known protein interactions (1). Using *InSpiRe*, we performed a meta-analysis of multiple microarray datasets from FSHD muscle biopsies, then removed secondary rewiring due to inflammation, aging and muscle wasting by using non-FSHD datasets.

Results: Using *InSpiRe* we generated an unbiased, unified network of interactions rewired in FSHD (1). We found that β-catenin was the main coordinator of FSHD signalling, with pathways including canonical Wnt, HIF1-α and TNF-α clearly perturbed. To detect transcriptional changes directly elicited by *DUX4*, gene expression profiling was performed on murine myoblasts. This revealed that *DUX4* significantly modified expression of the genes in our FSHD network (1). We are now experimentally testing pathways that we have identified, for their contribution to DUX4-mediated pathology and developing new mathematical tools to explore myogenic progression in FSHD from high-throughput time course imaging and RNA-seq data.

Conclusion: We provide the first unified molecular map of FSHD signalling (1), capable of uncovering pathomechanisms and guiding therapeutic development.

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(1) Banerji et al., *J R Soc Interface,* **12**(102), 20140797, 2015.

P07

Transcriptome and rescue therapy studies on mdx and C57BL/10 cardiomyocytes undergoing a hypertrophic response

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Background: Prospective therapies for Duchenne muscular dystrophy (DMD) must deliver functional restoration in both skeletal and cardiac muscle. Standardised methods for trialling these therapies in skeletal muscle have been established, whilst *in vitro* models of cardiomyocytes are still limited.
Aims: Here we present a method that has potential for being used as an assay for testing DMD therapies in cardiomyocytes.
Methods: Cardiomyocytes were isolated from mdx and C57BL/10 control mouse embryos. The hypertrophic effect of serum starving these cells was quantified by recording changes in area and volume measurements. RNA-Seq was performed on biological replicates of both disease model and control cardiomyocytes. Adeno-associated viruses were used to deliver a µDys construct to mdx cardiomyocytes and several pharmacological compounds were also used to investigate their potential for rescuing the hypertrophic response.
Results: C57BL/10 and mdx mouse cardiomyocytes exhibited a rapid hypertrophic response upon growth medium serum content reduction. Over the course of serum starvation this initial response was maintained in mdx, but not in control cardiomyocytes, leading to a more dramatic size increase in dystrophic cells. RNA-Seq identified pathways responsible for these differences, unravelling novel targets for potential therapies against the dystrophic phenotype in cardiac tissue. The hypertrophic response in mdx cardiomyocytes was attenuated with various compounds proposed as therapeutic agents in DMD.
Conclusion: Cardiomyocytes derived from mdx mouse embryos undergoing a hypertrophic response to serum starvation can be used as an outcome measure for *in vitro* assessment of the cardiac efficacy of DMD therapies.

P08

Investigating novel *COL12A1* mutations in a cohort of Bethlem like patients and its underlying pathology

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Collagen VI related myopathies are characterized with muscle weakness, hypotonia and connective tissue involvement. Mutations in *COL6A1*, *A2* and *A3* are responsible for only about half of collagen VI related myopathies. Recently, mutations in *COL12A1* have been reported in collagen VI related myopathies without *COL6A* mutations (1, 2). Collagen XIIA1 as a member of Fibril Associated Collagens with Interrupted Triple helices (FACIT) interacts with collagen I fibrils in the extracellular matrix (ECM) (3). Here, we report novel de novo *COL12A1* mutations in patients with a Bethlem phenotype and related pathologic mechanisms.
Two mutations in the gene *COL12A1*, COL12A1:g.C75815009T and COL12A1:c. C>5289A p.D1736E were identified in two patients with a genetically unresolved Bethlem phenotype. RT-PCR and in silico analysis confirmed heterozygous in frame deletion of *COL12A1* exon 54(c.del:8345-8431het) in one of the patients, a conserved region in the collagenous domain of the protein. Immunofluorescence studies in dermal fibroblasts revealed intracellular retention of collagen XII and immunoblotting showed an increase in colXIIA1 protein in culture medium of patient fibroblasts. Transmission electron microscopy and immunogold staining was applied to investigate collagen fibre pattern and diameter in mutant cultured fibroblasts and in vitro extracted collagens. The findings suggest an impaired assembly and secretion of colXIIA1 protein in mutant cells. Coculture experiments of mutant fibroblasts with C2C12 revealed a significant decrease ($P \leq 0.05$) in myoblast fusion index in mutant cultured fibroblasts which suggests a role for collagen XII in myoblast differentiation. Our findings emphasize the role of collagenXII mutations in patients with muscle weakness and connective tissue involvement. In addition, the phenotypic spectrum of extracellular matrix myopathy due to *COL12A1* mutations has been extended.
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P09

Establishing *in vitro* and *in vivo* outcome measures in a pre-clinical treatment study for Duchenne muscular dystrophy with an inhibitor of the Na+/H+ exchanger 1

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The absence of dystrophin in Duchenne muscular dystrophy (DMD) muscle cells results in increased membrane permeability and subsequent intracellular calcium overload. The dysregulation of calcium homeostasis that characterizes the DMD models is exacerbated by the increased activity of the Sodium/Hydrogen Exchanger 1 (NHE1). NHE1 overactivity leads to an increased influx of sodium, which in turn switches the Sodium/Calcium Exchanger (NCX) into reverse mode, resulting in an increased calcium influx. Selective NHE1 inhibitors can be used to reduce the sodium influx and thereby revert the NCX to normal mode with a subsequent decrease in the cellular calcium load. This observation has led to the hypothesis that the use of specific NHE1 inhibitors could improve the calcium homeostasis and alleviate pathology in DMD muscle. The current study seeks to demonstrate the efficacy of a specific NHE-1 inhibitor that has a good safety and potency profile in several pre-clinical studies. During the first part of the study, an *in vitro* calcium-flux assay using Fluo-4 will be used to verify the drug action and to determine the optimal dose required to study the efficacy in long term chronic studies. For the calcium assay, isolated muscles will be enzymatically and physically disaggregated and single fibres will then be loaded with a fluorescent calcium sensing dye measuring calcium influx when external free calcium levels are increased. Fluorescence over time, measured using a fluorescence microplate reader, will then be used to calculate real-time influx kinetics. For the second part of the study mdx mice treated chronically with drug in chow will be monitored *in vivo* by manganese enhanced magnetic resonance imaging (MEMRI) for calcium uptake in both skeletal and cardiac muscle. The imaging study will be supported by functional muscle testing and tissue analysis. The proposed studies with a first prototype NHE-1 inhibitor are an important step towards potential clinical trials for dystrophinopathies with this class of compounds.

P10

Molecular modelling and dynamic of beta-Sarcoglycan

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Background: Sarcoglycans (SGs) are transmembrane glycoproteins forming a complex with the dystrophin glycoprotein complex. The SG complex assembly follows a specific pathway where the formation of β/δ-SG core is crucial for its targeting to the sarcolemma. The SGs consist of an intracellular domain, a transmembrane region and an extracellular domain containing highly conserved cysteine residues. Structural characteristics suggest that the SG complex may be involved in signal transduction from the extracellular to the intracellular region. Due to

their interaction with other crucial proteins and involvement in muscular dystrophies, solution of their structure is an important issue.
Aims: Our aim was to obtain the 3D structure of β-SG using a bioinformatics approach since this has not yet been resolved by X-ray crystallography or NMR spectroscopy.
Methods: A PSI-BLAST analysis did not reveal homologous proteins of known structure. We used a fold recognition algorithm to obtain a 3D model of β-SG. Disulfide bonds between Cys288-Cys314 and Cys290-Cys307 were used as restraints. Three N-glycosilation sites were located in the extracellular domain. A 20ns molecular dynamics simulation was performed for the structure embedded in lipid bilayer and solvated by explicit water molecules to study the structural and dynamic properties of β-SG.
Results: Analysis of β-SG secondary structure showed β-strands organised in β-hairpin motifs in the extracellular domain and an α-helix in the transmembrane region. The root-mean square deviation was calculated for the C_α-atoms of β-SG shows that the β-strands structures are stable with a low flexibility. The loop formed by the residues 266-276 has the highest mobility.
Conclusion
Using a bioinformatics approach we obtained a 3D model of β-SG; however further studies are needed to verify the reliability of this 3D model. The determination of the SGs 3D structure may provide useful information on the function of the SG complex itself and its interaction within the dystrophin glycoprotein complex.

P11

Heterozygous CAPN3 patients: a review of our cohort to direct further investigation

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Background: Limb Girdle Muscular Dystrophy type 2A (LGMD2A) is a recessive muscular dystrophy caused by defects in the *CAPN3* gene, encoding for the muscle specific protease calpain3. The disease is characterised clinically by a specific pattern of weakness and three cardinal features: scapular winging, contractures and normal respiratory function. LGMD2A patients may show altered calpain3 staining on western blot, although abnormal calpain3 staining is not specific to LGMD2A. Patients carrying a single mutation are often phenotypically similar to LGMD2A patients, yet no second mutation in *CAPN3* can be found by conventional sequencing.
Aims: To review our cohort of undiagnosed patients heterozygous for a *CAPN3* mutation in order to select cases for further *CAPN3* investigation, such as by MLPA and mRNA sequencing.
Method: 19 patients were identified. 15 carried a single pathogenic mutation. An additional 4 with a *CAPN3* variant of unknown pathogenicity were included because calpainopathy was suspected clinically. Medical, physiotherapist's assessments and western blot pattern were collated and compared with previously reported disease characteristics.
Results: Of 19 patients, only 2 had all three cardinal clinical features. 9/15 of the available western blots showed reduction of calpain3. One subgroup of three families, with mutation (c.643_663del), showed a distinct phenotype with facial involvement, fatigability and an apparent dominant inheritance pattern. They had no contractures, only mild scapular winging and a milder weakness than other heterozygous and LGMD2A patients.
Conclusion: Phenotypic variability is present in heterozygous patients, some patients show phenotypic and biopsy findings in keeping with LGMD2A while others are markedly different. Patients fitting the characterisation may benefit from further *CAPN3* investigation. The specific features of the c.643_663del patients are particularly interesting, suggesting either a different diagnosis or alternative pathogenic mechanism.

P12

Combination antisense treatment for Duchenne muscular dystrophy : open reading frame rescue of dystrophin in conjunction with destructive exon skipping of myostatin in neonatal *mdx* mice.

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The fatal X-linked Duchenne muscular dystrophy (DMD), characterized by progressive muscle weakness and muscle wasting, is caused by mutations within the dystrophin gene. One of the strategies for DMD treatment that is currently in a phase III clinical trial is antisense oligomer (AO)-induced exon skipping to restore the dystrophin gene reading frame, subsequently generating a truncated but functional protein. In addition, down regulation of myostatin exon, a negative regulator of skeletal muscle growth and differentiation, through AO-induced destruction of the myostatin gene reading frame has been shown to improve muscle size and muscle mass together with a reduction of fibrosis in experimental animals.
In regards to this, we have recently demonstrated that dual exon skipping of dystrophin and myostatin genes induced by peptide-conjugated phosphorodiamidate morpholino oligomers (BPMOs) provided therapeutic effects in locally injected muscles of adult *mdx* mice, the animal model of DMD. Following this encouraging result, here we reinvestigate the combined treatment, but systemically through an intraperitoneal administration of BPMOs, into neonatal *mdx* mice. The BPMOs were injected into the neonates on the day of birth, followed by two additional injections at weeks 3 and 6. We observed in treated animals (compared to age-matched saline injected controls) an improvement in locomotor behaviour, normalisation of muscle mass in associated with enhanced dystrophin expression and reduced regeneration in the diaphragm in particular. Our data strongly supports the use of AO-induced exon skipping for the treatment of DMD and for other muscle wasting diseases, although further optimisation of a dosing regimen needs to be addressed for maximal therapeutic benefits.
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P13

STIM1 mutations at a common amino acid residue (p.340) identified in two individuals with a predominant muscle disease phenotype

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Background: Dominant mutations in STIM1 have been identified in the complex phenotype Stormorken Syndrome (SS) and in non-syndromic tubular aggregate myopathy (TAM). To date all individuals with SS have a common p.R304W gain of function mutation in the coiled coil domain 1 of STIM1. In contrast mutations in patients with STIM1-related TAM are restricted to the EF hand domain.
Aims: To identify and characterise patients with mutations in STIM1.
Methods and Patients: We performed whole exome sequencing on patient 1 and identified a *de novo* STIM1 mutation. We subsequently selected 4 patients with tubular aggregates, and 4 patients selected by phenotype,

and performed immunostaining for STIM1 and direct sequencing of the STIM1 gene. Patients with STIM1 mutations were deep phenotyped, including investigation for SS features.
Results: Two patients with STIM1 mutations were identified. Patient 1 has the common SS mutation (p.R304W), and exhibits features in keeping with this (thrombocytopaenia, miosis, evidence of hyposplenism and hypocalcaemia). Tubular aggregares were present in the muscle biopsy and showed accumulation of STIM1 Patient 2 has a novel mutation at the same amino acid residue (p.R304G), and presents with a strikingly similar pattern of muscle symptoms and signs but aside from miosis no additional features of SS. The neuromuscular phenotype in both patients comprises myalgia, muscle stiffness, and reduction in range of joint movement, with mild weakness on examination.
Conclusion: The use of STIM1 immunoananlysis in patients with tubular aggregates was successfully applied to screen for patients with STIM1 mutations. We report a novel mutation at the common SS amino acid residue in a patient with TAM and miosis, in addition to further characterisation of a new patient with SS. The neuromuscular phenotype of both patients is similar and recognisable, and given the potential associated features, making this diagnosis has implications for clinical care.

P14

Clinical outcomes in a large cohort of boys and adolescents with Duchenne muscular dystrophy .

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Background: Standards of care including corticosteroid and non-invasive ventilation (NIV) have significantly changed the course of DMD and improved survival so that now transition into adult care is common.
Aim: To describe the morbidity and mortality in a large pediatric DMD population with contemporary care and identify the milestones of disease progression
Methods: The Dubowitz Neuromuscular Centre offers diagnosis and management of DMD through the childhood years, with transition to adult teams at age 17 years. Retrospective analysis of this DMD cohort was undertaken. Patients were identified from existing databases and disease course, medical interventions, and outcomes were collated .
Results: Over the past 20 years, 310 DMD patients, born between 1989 -2013 were seen, with 18±6 sequential assessments for the individual patient. Overall, 63% lost ambulation at a mean age of 10.4±1.9 years in the corticosteroid treated group, compared to 9±1 in the steroid naïve group.
231/310 were older than 10 years, and in this subgroup, 50% developed scoliosis, 20% use NIV (age at initiation 14.3±2 years) and 51% have echocardiographic cardiomyopathy (age at onset 12.3 ±2.9 years). All patients with cardiomyopathy were treated with ACE inhibitor ± beta-blocker.
93 patients were transitioned to adult service. At the time of transition, 57% had FVC <60%, 32% were on NIV, 85% had cardiomyopathy, and 26% were on corticosteroid treatment. 10/93 were on tube feeding.
20 patients died at 14.9±2.5 years. Death was cardiac-related in 6, sudden in 2, pneumonia in 5 (3 on NIV), gastrointestinal complications in 2, and perioperative in 2.
Conclusion: Despite the improved survival, there still is significant mortality in DMD in the childhood years. Cardiomyopathy was the most frequent cause of death, and gastrointestinal related mortality also plays an important role. These data will help direct care to reduce mortality and has implications in development of adult services.

P15

The frequency and characterisation of cardiac involvement in female carriers of BMD or DMD: a cross sectional analysis

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Background: Duchenne and Becker muscular dystrophy (DMD/BMD) are X-linked, recessively inherited disorders resulting from a deletion within the dystrophin gene. Female Xp21 carriers often are asymptomatic but at risk of cardiac dystrophinopathy. Carriers have an estimated 7-18% incidence of dilated cardiomyopathy and it is recommended that all should be offered cardiac surveillance.
Aims: The aim was to determine the incidence and severity of left ventricular (LV) dysfunction as detected by echocardiogram (ECHO) within a large cohort of female carriers of DMD/BMD.
Methods: Following diagnosis of relatives with DMD/BMD, all relevant family members are offered carrier testing. A registry of all carriers is maintained by the *Neuromuscular Genetics service.* All patients are offered cardiac screening; consisting of symptom review, 12-lead ECG and 2D ECHO. All patient notes were included from October 1999 to July 2014 (n=130) and were retrospectively reviewed for ECHO assessments including measurement of LV end-systolic, end-diastolic dimensions, wall thicknesses and fractional shortening (FS). LV ejection fraction was measured in a variety of ways. Some patients had tissue-Doppler assessments of LV segmental function. Diastolic measures of cardiac function were measured.
Results: No patients had cardiac symptoms. Mean age at screening was 39 years (range 5-81 years). 12.6% of patients had a FS of less than normal (<28%), 1.7% of patients had <40% LVEF (corrected) and 33% of patients had <55% LVEF (uncorrected). 11 patients (8.5%) had wall motion abnormalities. No correlation was found between age, CK, or muscle symptoms and LV dysfunction.
Conclusion: Incidence of cardiomyopathy is between 1.7% and 33% depending on definition. 'Uncorrected' LVEF is likely to overestimate systolic dysfunction whereas presence of wall motion abnormalities is most specific for cardiac dystrophinopathy in carriers without comorbidities. No correlation is seen between age, CK levels, and LV dysfunction; possibly suggesting that there may be a trigger to LV dysfunction.

P16

Polycomb group genes in human neuromuscular diseases.

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Polycomb group (PcG) proteins are transcriptional repressors that remodel chromatin through epigenetic modifications. In mouse models, two PcG proteins, Bmi1 and Ezh1/2, have been shown to play a crucial role in skeletal muscle regeneration through regulation of self-renewal and proliferation of satellite cells.
In an mdx context, conditional overexpression of Bmi1 in satellite cells of adult muscles enhances their regenerative capacity leading to strikingly improved muscle strength (Di Foggia et al JExpMed, 2014).
We hypothesise that modulation of Bmi1 expression in human muscle progenitor cells could be exploited therapeutically to enhance the regenerative potential of the skeletal muscle in conditions of chronic muscle wasting.
Here we have characterised the expression of Bmi1 and other PcG proteins, in quiescent and activated satellite cells, in a wide spectrum of chronic neuromuscular disorders, including inflammatory myopathies (n=4), DMD (n=8) and MND (n=4) as compared with age-matched controls. We show that Polycomb group proteins are expressed in the human muscle during the regenerative process, however a significant reduction of their expression is observed in both quiescent (Pax7+) and activated (Pax7+;Myf5+) satellite cells of patients with

chronic neuromuscular disorders, irrespective of their aetiology. Interestingly, a similar expression pattern was found also for Ezh1.
Lentiviral-mediated overexpression of Bmi1 in short term cultures of human myoblasts isolated from DMD patients and from age matched controls increased the proliferation of the myoblasts (EdU pulse followed by immunostaining) and enhanced their differentiation (IF for MyHC and fibre size analysis) in vitro. Elucidation of the molecular mechanisms underpinning this phenotype in human myoblasts is ongoing and the results of this analysis will also be presented.

P17

Prevention of exercised induced cardiomyopathy following Pip-PMO treatment in dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is a severe, fatal muscle wasting disorder, caused by mutations in the gene encoding the structural protein dystrophin. In addition to the skeletal muscle phenotype, DMD patients develop cardiomyopathy, which significantly contributes to mortality. Antisense oligonucleotides (AOs) are a promising therapy for DMD, acting to restore functional dystrophin protein by exon skipping. However, a major limitation of current AOs is the absence of dystrophin correction in heart. We have previously described second generation peptide-AO conjugates, which dramatically improved dystrophin protein restoration in muscle, including cardiac muscle, of the murine DMD model, *mdx*. To determine the clinical benefit of these peptide-AOs on cardiac function, *mdx* mice were subject to forced exercise to closely model the DMD cardiac phenotype. Repeated intravenous peptide-AO doses resulted in high levels of exon skipping and dystrophin protein in heart, and which prevented onset of cardiomyopathy, with normalised heart size and left and right ventricular cardiac outputs as determined by cine-MRI. Treated mice exhibited stabilised *Nppa* levels, a marker of haemodynamic overload, significant reduction in cardiac fibrosis and improved cardiac sarcolemmal integrity. This study therefore demonstrates that high levels of cardiac dystrophin restored by peptide-AOs prevent development of cardiomyopathy and reduce heart pathology in a clinically relevant cardiac mouse model of DMD. Thus early intervention to restore cardiac dystrophin may prevent DMD cardiomyopathy and similar AO interventions may have clinical benefit in related familial cardiomyopathies.

P18

Assessing cognitive ability in mouse models of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a neuromuscular disease arising from mutations in the dystrophin-encoding *DMD* gene. Aside from muscle pathology, cognitive impairment is also a prominent aspect of the disorder, with approximately one third of DMD boys possessing a degree of cognitive deficit, frequently manifesting itself in memory impairment. Within the brain, dystrophin is abundantly expressed in pyramidal neurons of the hippocampus. The dystrophin deficient *mdx* mouse and an *mdx* strain also null for the Cmah gene (Cmah-/-mdx), which is similarly mutated in humans, served to analyse the effect of dystrophin deficiency on

cognitive performance.
Aims: To investigate the effect of dystrophin loss on cognitive functioning, particularly the effect on hippocampal dependent memory in DMD mouse models.
Methods: Barnes Maze testing; a task that measures spatial learning and memory aspects of cognition, was performed on control (n=4), *mdx* (n=4) and *Cmah-/-mdx* (n=4) mice aged between 44-47 weeks. Testing included an adaptation phase, a spatial acquisition phase consisting of four consecutive training days with four trials per mouse per day (days 1-4) and two reference memory probe trials; including short-term memory (day 5) and long-term memory (day 12). Probe trials were assessed for primary latency, primary errors, total errors and the preference for holes around the maze. A success score was created for hole preference. This was the number of head pokes in a hole, multiplied by a given value (based on orientation around target hole) for that hole.
Results: On average mutant mice showed increased total latency and an increased number of errors for finding the target hole. Control mice employed a direct pattern of searching whereas mutant mice utilized a mixed search strategy. Anxiety related behavior was highest in the *mdx* mice during test days.
Conclusion: Based on the Barnes Maze testing short-term and long-term hippocampal memory seems to be impaired in DMD mouse models.

P19

Biomarker development to support clinical development of utrophin modulation for Duchenne muscular dystrophy therapy.

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Background: Utrophin modulation has therapeutic potential for Duchenne muscular dystrophy (DMD) regardless of the dystrophin mutation. To demonstrate efficacy of utrophin modulators, it is essential to develop robust and reproducible protocols to quantify utrophin and other biomarkers within patient muscles. We hypothesise that utrophin sarcolemmal maintenance within a dystrophin-deficient myofibre would protect it from necrosis and consequently there would be significantly less muscle regeneration within treated muscles. However utrophin is expressed at higher levels in regenerating muscle compared to mature muscle fibres so this must be taken into consideration during analysis.
Aims: Our aim is to quantify sarcolemmal utrophin in mature and regenerating fibres and to quantify the number of regenerating muscle fibres in muscle biopsies from DMD, Becker muscular dystrophy (BMD) and control muscle.
Methods: Fluorescence immunostaining followed by image analysis was performed to quantify utrophin intensity. To identify newly regenerating fibres, a cocktail of antibodies to foetal and developmental myosin antibodies was used.
Results: Our data confirm that, in general, muscle biopsies from BMD patients have lower numbers of recently regenerated fibres and overall reduced utrophin intensity compared to biopsies from DMD patients.
Conclusions: Development of these new methods provide sensitive and robust tools to quantify sarcolemmal utrophin and muscle regeneration from muscle biopsies. These tools will be invaluable for confirming utrophin modulator activity in future DMD clinical trials.

P20

MicroRNAs as biomarkers to monitor disease severity and response to antisense oligonucleotide therapy in spinal muscular atrophy

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Background: Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality, resulting from homozygous deletion in the Survival Motor Neuron gene 1 (*SMN1*). MicroRNAs (miRNAs) are a class of small (~22nt) endogenous non-protein-coding RNA molecules that post-transcriptionally regulate gene expression. miRNAs have been shown to play an important role in the regulation of muscle and nervous system development.
Aims: In this study we aim to: 1) identify the dysregulation of some selected miRNAs in SMA; 2) determine the correlation between candidate miRNAs expression and disease severities both in SMA mouse models and patients; and 3) investigate the response of candidate miRNAs to experimental antisense oligonucleotide (AON) therapy in SMA mice.
Methods and Materials: MicroRNA miR-9, miR-206 and miR-132 have been selected based on their reported functions in motor neurons and skeletal muscle development and their correlation with the pathogenesis of SMA. The expression of miRNAs was determined in two different SMA transgenic mouse models, the severe type I and mild type III mouse models, and compared with the unaffected littermate controls. This was followed by studies on their response to effective antisense oligonucleotide therapy. miRNAs were measured in spinal cord, skeletal muscle and serum samples from SMA mice. The expression of miRNAs in serum was further evaluated in SMA patients with type II and type III phenotypes.
Results: Significant changes were observed in all three miRNAs in the spinal cord and skeletal muscle samples from severe type I mice compared with the unaffected littermate controls. The level of some dysregulated miRNAs was normalised after effective AON therapy in SMA mice.
Conclusion: Our study suggests that miRNAs could be used as biomarkers in SMA. The investigations on functional pathways of the informative miRNAs in SMA are underway.

P21

Developing allele-specific silencing therapeutic approach using antisense oligonucleotide on Collagen 6 genes in congenital muscular dystrophy

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Background: Allele-specific silencing by antisense oligonucleotide (AON) has recently been investigated in some neurodegenerative genetic diseases and has shown its therapeutic potential. This strategy is potentially applicable to dominant genetic disease in which haploinsufficiency is not pathogenic. The severe collagen VI-related congenital muscular dystrophy (CMD) variant, known as Ullrich CMD (UCMD), is caused by either recessive or dominate mutations in one of the three collagen 6 genes (COL6A1, COL6A2 and COL6A3). The fact that 50% of mutations are de-novo dominant, and that haploinsufficiency is not associated with clinical phenotypes in collagen 6 genes, provides the theoretical basis for using AON to selectively silence the mutant allele as a therapeutic strategy.
Aims: To develop a novel AON approach for collagen VI-related UCMD by using either the out-of-frame exon skipping strategy or RNase H cleavage strategy for allele-specific silencing.
Methods and Materials: Skin fibroblast established from UCMD patients carrying dominant mutations in collagen 6 genes are used as cellular model in this study. AONs targeting either the specific mutations or the common SNPs are synthesized in phosphorodiamidate morpholino oligomer (PMO) or as gapmer using the

chimeric 2'-O-methyl phosphorothiate (2-OMe) and phosphorothiate DNA chemistries. Allele-specific reverse-transcript PCR is used to amplify the product and to assess the efficiency of allele-specific silencing. Functional studies on collagen 6 after AON treatment in the fibroblast model are currently being assessed.
Results: In our preliminary data Gapmer AON displayed higher efficiency than PMO, in a dose-dependent manner, on allele-specific silencing *in vitro* when targeted the specific mutations. We are currently in the process of evaluating the functional outcome of some Gapmers on collagen VI in UCMD fibroblast.
Conclusion: Gapmer AONs have shown the potential in selectively silencing the mutant allele in collagen 6 gene *in vitro* in cellular models. Further improvement on the design of Gapmer to target common SNPs is still required for the wider application of this strategy in the therapy of UCMD.

P22

The National Diagnostic and Advisory Service for limb-girdle muscular dystrophies in Newcastle

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A diagnostic service for Rare Neuromuscular Disorders was established in 2001 as a consortium of four centres in the UK to advance diagnostics in such diseases. Newcastle is the national referral centre for the diagnosis of limb-girdle muscular dystrophies (LGMDs), providing a nationally funded service for patients referred from England, Scotland and Wales. LGMDs are caused by at least eight genes with dominant inheritance and over twenty genes with recessive inheritance. Patient diagnosis is challenging due to the overlap in clinical presentation not only among different LGMD subtypes but also with other forms of muscular dystrophy. Diagnosis is ultimately resolved by identifying the genetic defect, and protein analysis of muscle biopsies is a useful test for identifying or excluding a significant number of these dystrophies. In Newcastle we offer multidisciplinary assessment of LGMDs with specialist clinicians, physiotherapists and comprehensive laboratory diagnostics provided at both the protein and DNA level. A research and development programme is also in place to further characterise newly discovered disease-causing genes and their phenotypical spectrum. Our approach is beneficial to patients as the specialised diagnostic expertise is concentrated in one centre leading to more effective diagnoses and management.

P23

Evaluation of oral small molecules which modulate expression of utrophin and ameliorate pathology in the *mdx* mouse

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Background: Duchenne muscular dystrophy (DMD) is a devastating muscle-wasting disease caused by lack of the cytoskeletal protein dystrophin. There is currently no cure for DMD although various promising approaches are progressing through clinical trials. In partnership with Summit plc, we previously developed SMT C1100, an oral small molecule utrophin modulator that reduces dystrophic symptoms in the *mdx* mouse and completed a Phase 1a healthy volunteer trial. Results from a recent Phase 1b study of SMT C1100 in DMD patients, showed it to be

safe and well tolerated. Also observed was a reduction in plasma levels of enzymes associated with muscle damage which are typically elevated in DMD patients.
Aims: By pharmacologically modulating the dystrophin-related protein utrophin, we are developing a therapy applicable to all DMD patients, regardless of their mutation in dystrophin. SMT C1100 is a first-in-class utrophin modulator; we are exploring future generations of utrophin modulators to find the best-in-class molecules.
Methods: We identified multiple new classes of compounds which modulate utrophin expression in both mouse and human DMD myoblasts using a high throughput luciferase reporter bioassay. Compounds demonstrating an increase in utrophin levels were then tested *in vivo* on the dystrophin deficient *mdx* mouse. Histological analysis, western blots, bioassays, immunofluorescence and physiological tests were used to determine utrophin levels and if the dystrophic phenotype was improved in muscle and heart.
Results: Several next generation molecules demonstrated positive effects following daily oral administration in the *mdx* mouse. The increases in utrophin levels along with beneficial downstream changes in skeletal muscle, diaphragm and cardiac pathology will be presented.
Conclusion: These results demonstrate that utrophin modulation can significantly reduce the dystrophic phenotype in all muscle types including the diaphragm and heart. This validates the concept of maintaining utrophin expression in muscle as a potential means to treat all DMD patients.

P24

Expression of truncated telethonin in a patient with limb-girdle muscular dystrophy 2G

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Background: Telethonin is a sarcomeric Z-line protein encoded by the *TCAP* gene. Non-sense mutations in *TCAP* cause the rare autosomal recessive limb-girdle muscular dystrophy type 2G (LGMD2G). The disease is characterized by onset in the first/second decade of life with proximal/distal weakness in the legs and proximal weakness in the arms. Loss of autonomous ambulation occurs by the fourth decade. Cardiac involvement is frequent and the serum ***CK*** levels can be slightly too significantly raised. Deficiency of telethonin in a muscle biopsy is considered a highly specific diagnostic clue for LGMD2G.
Aims: To investigate the expression of telethonin in a patient with a novel homozygous mutation, c.244C>T (p.Gln82X) in the *TCAP* gene.
Patient: A 49 year-old male patient presenting with a classical LGMD phenotype, born from non-consanguineous healthy parents of Indian descent. He had normal motor milestones, becoming slower in his teens and presenting scapular winging and Achilles tendon contractures. He became wheelchair-bound and unable to lift his arms to shoulder height by age 44. His serum ***CK*** activity was elevated up to 9000 IU/l. Cardiac investigations did not reveal any abnormalities.
Results: The muscle biopsy showed myopathic features and absence of labelling with an antibody to the C-terminal portion of telethonin. However, an antibody directed against the full-length protein showed labelling on sections and a single band of ~10 kDa on immunoblot, consistent with the predicted size of the mutant protein.
Conclusion: In contrast to observations in all LGMD2G cases reported to date, expression of mutant protein was detected in our patient. Our results suggest that there may be patients where a diagnosis of LGMD2G has been overlooked due to the retention and detection of telethonin expression, albeit a mutant protein.

P25

Pathophysiology of anoctaminopathy (LGMD2L)

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Background: Anoctamin 5 (ANO5), suggested to function as a calcium activated chloride channel, is the causative gene underlying LGMD2L and Miyoshi myopathy type 3 (MMD3). These 'anoctaminopathies' are the third most common muscular dystrophies in the North of England. Like all LGMDs, LGMD2L is characterised by weakness of the shoulder and hip musculature, generally increasing in severity with age.
Aims: To understand the subcellular localisation of ANO5.
Methods: A myc-tagged ANO5 construct was co-transfected into undifferentiated C2C12 with plasmids expressing GFP-tagged organelle specific markers for endosomes, endoplasmic reticulum, Golgi apparatus and dysferlin. The day after transfection, differentiation was induced for 5 days to allow for the formation of myotubes before IF staining to detect the myc tag.
Results: Co-localisation data of myc-tagged ANO5 with organelle specific markers suggests that anoctamin 5 localises to the endosomes in both differentiated and undifferentiated C2C12s. There is also some evidence to suggest that ANO5 partially co-localises with BIN-1, a marker of T-tubules, and dysferlin.
Conclusion: The use of tagged constructs in localisation studies is a valuable resource in further investigation of the location and function of ANO5, especially in the absence of a specific primary antibody for this protein. The localisation of ANO5 in the endosomal compartment and overlapping both dysferlin and a marker of T-tubules suggests further routes of investigation into the function of ANO5 and the pathophysiology of LGMD2L.

P26

Repurposed cancer therapeutics as treatments for DMD

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Loss of the dystrophin protein due to mutations in the *Dmd* gene causes Duchenne muscular dystrophy (DMD), a severe muscle wasting disease affecting roughly 1 in every 3600 male births. The loss of dystrophin also leads to the loss of the dystrophin glycoprotein complex (DGC); in normal muscle the DGC stabilises the sarcolemma by making regularly spaced connections between the fibre cytoskeleton and extracellular matrix. By studying the dystrophin glycoprotein complex in DMD we have previously identified tyrosine phosphorylation of dystroglycan, a core component of the DGC, as a key signal in the degradation and loss of the DGC from the sarcolemma. Preventing phosphorylation of dystroglycan in the *mdx* mouse model of DMD by a targeted gene knock-in of phenylalanine at tyrosine residue 890 ameliorates the dystrophic phenotype. Studies in mouse myoblasts also demonstrate pharmacological intervention with proteasome or tyrosine kinase inhibitors increases levels of non-phosphorylated dystroglycan. Furthermore, preventing dystroglycan degradation with tyrosine kinase or proteasome inhibitors, which are already approved for clinical use as cancer therapeutics, rescues the dystrophic phenotype and improves swimming ability in *sapje* zebrafish, a fish model of DMD. To further investigate the efficacy of small molecule inhibitors as potential therapeutics for DMD, tyrosine kinase or proteasome inhibitors were administered to 5 week old male *mdx* mice for 2 weeks, thus allowing the peak period of muscle degeneration and regeneration in this model to be targeted. Treatment with both classes of inhibitor led to improved muscle pathology, restoration of DGC components to the sarcolemma and improved wire hanging times. Previous studies, together with preliminary data from our ongoing experiments with the *mdx* model, demonstrate that preventing tyrosine phosphorylation in dystroglycan may provide a valuable therapeutic intervention for DMD.

P27

Development of a novel approach using CRISPR/Cas9 nucleases to correct duplications in the dystrophin gene.

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Background: Duchenne muscular dystrophy (DMD), caused by mutations in the *DMD* gene, is the most common inherited muscular dystrophy. DMD patients suffer progressive weakening of muscles, and ultimately heart and respiratory failure, leading to premature death. **Aims:** Here we propose a genome editing method to remove duplications in the *DMD* gene (cause of ~10% of DMD cases). CRISPR/Cas9 nucleases insert a DNA double strand break (DSB) in a specified region of the genome. By targeting nucleases to duplicated intronic regions of dystrophin, we aim to produce two DSBs flanking one copy of the duplicated region, leaving the cell to repair the DSBs by non-homologous end joining, removing the duplication.
Methods or Patients or Materials: CRISPR/Cas9 nucleases have been introduced by transfection as well as integrated into a viral vector for delivery into HEK293 cells and patient-derived fibroblasts. **Results and Conclusion:** We have identified three nucleases that target with high efficiency the *DMD* gene. Current work is aimed at establishing a viral expression system and determining the efficiency in repair of duplications in DMD-derived cell lines.

P28

Testosterone therapy in Duchenne muscular dystrophy

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Background: The health of adolescents with DMD has improved greatly as a result of corticosteroid use and many now seek to lead independent adult lives and relationships. Unfortunately, steroids have a significant impact on growth, and pubertal delay is almost universal. If muscle mass/strength and bone density can be maximised during puberty it may allow boys with DMD to remain ambulant for longer. Despite this, there are no guidelines or consensus for the use of testosterone in delayed puberty associated with DMD.
Aim: To retrospectively determine pubertal response and associated growth of adolescents with DMD and pubertal delay who were treated with testosterone.
Methods: Data was analysed from boys with DMD who were treated between 2008 and 2014. Anthropometric and pubertal data was retrieved from case notes. Sex and age adjusted Height z-scores were calculated.
Results: 14 boys in Newcastle were treated; median age at start 14.5 years. 8 have finished (mean treatment period 3.1 years), 6 are still on treatment. Mean testicular volume pre-treatment was 2.4 mls, post 3.9 mls. Mean initial testosterone level was <1.0 nmol/l, final 5.4 nmol/l. At start of testosterone therapy mean height z-score was -4.32 compared to mid-parental of 0.35, by the end -4.4. Mean height velocity increased from 0.48 cm/year before treatment, to 4.7cm/year. Mean height gain was 14.2 cm (range 11.0-19.0 cm)
Conclusion: Testosterone appears to be useful and well-liked but there is a large variation in regimens and response s and the usual growth increment from puberty appears compromised. Few had adult endogenous testosterone levels after treatment; we need to continue follow-up as prolonged treatment for hypogonadal hypogonadism may be required. Mean age at treatment initiation was >2 years after we expect natural pubertal development. Testosterone may improve muscle and bone density in this cohort at a critical time, if treatment is

initiated earlier. Controlled studies are required to establish the most appropriate treatment regimen and determine the precise impact on key outcomes such as muscle function.

P29

Care provision for adults with Duchenne muscular dystrophy in the UK: compliance with international consensus care guidelines

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Background: We present results from the CARE-NMD survey of Duchenne muscular dystrophy (DMD) highlighting care received by adults (n=201). Adult care practices in the UK were compared to those for UK children, and those for adults in Germany, Denmark, and Eastern Europe.

Aims: To investigate the extent to which care practices met international consensus guidelines, identify disparities in care, and understand reasons for non-compliance.

Methods: A 42-question self-report survey, distributed via TREAT-NMD National DMD Registries in seven European countries and based on consensus care guidelines, captured data on activities of daily living, functional abilities, disease progression, and medical and social care.

Results: Of 1,062 responses, 42 were from UK adults (mean age 24.8 years). Total adult non-attendance at specialised neuromuscular clinics was the highest of any country surveyed (22%), and considerably higher than UK children (3.4%). UK adults reported lower satisfaction than children or adults elsewhere in Western Europe. Care provision does not meet international consensus guidelines for a significant proportion of these patients. Only 42.9% received both lung function tests and echocardiogram at-least annually; 19% received neither. Frequency of these essential assessments was significantly associated with regular attendance at specialised clinics.

Adult care was less comprehensive than paediatric care, with a drop in frequency of cardiac and respiratory assessment noted at transition. Adults were less likely to have regular functional ability assessments or to receive professional physiotherapy (21.4%) than children (55%). UK adult and child access to professional physiotherapy was the lowest of all countries surveyed.

Social inclusion was also poor, with the highest proportion of adults living with their families (92.9%) in Western Europe, and the lowest proportion living independently or participating in employment (none) or education (25.6%)

Conclusions: These data highlight shortcomings in care for UK adults with DMD compared to children, and adults elsewhere in Europe.

P30

Longitudinal characterisation of cardiac function in the *Cmah-/-mdx* mouse model of DMD

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Background: The *mdx* mouse is the most well characterised animal model of DMD and is the subject of nearly two and a half thousand publications to date. However, the natural history of pathology in *mdx* is somewhat different to that of patients and is less severe, particularly with respect to cardiac phenotype.
The CMAH (cytidine monophosphate-sialic acid hydroxylase) gene is naturally deleted in humans and introduction of a human-specific deletion of this gene in *mdx* or *alpha-sarcoglycan*-deficient mice has been reported to result in increased muscle pathology, and models which represent the patient condition more faithfully. There is however currently no functional characterisation of the cardiac phenotype of these mice.
Aims: We aimed to characterise the cardiac phenotype of *Cmah-/-mdx* mice and to assess if they are more severe than *mdx* mice and hence a more useful model of DMD.
Methods: We assessed the heart function of male *Cmah-/-mdx* mice at 12, 18 and 28 weeks by cardiac MRI and at 28 weeks by conductance catheter, alongside *C57BL10* wild type controls and *mdx* mice. Following functional assessment at 28 weeks, hearts were harvested for histology.
Results: We present evidence which suggests *Cmah-/-mdx* mice have an earlier onset of cardiac dysfunction than *mdx* mice however differences between the strains are less striking by 28 weeks.
Conclusion: Although cardiac dysfunction is evident at an earlier age in *Cmah-/-mdx* compared to *mdx* mice, they were less severely affected than expected by 28 weeks of age. It is unclear if this model holds any significant benefits over the *mdx* mouse.

P31

Vitamin D in corticosteroid treated Duchenne muscular dystrophy : what dose achieves serum 25OH vitamin D sufficiency?

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Background: Vitamin D status in DMD has been addressed in dedicated workshops over the years. However, there is lack of consensus as to the appropriate dosing requirements.
Aim: Assessment of the efficacy of various vitamin D doses to attain sufficiency level.
Method: 25-OH vitamin D levels & concurrent vitamin D dose were collected form retrospective case-note review of DMD patients at Dubowitz Neuromuscular Centre. Vitamin D levels were stratified as deficient <50 nmol/l, insufficient 50-75 nmol/l, and adequate >75nmol/l.
Result: 617 levels were available from 197 patients (patients tested 1–7 times). Median age at level estimation was 9.7 years (2-18). 90% levels were performed whilst on corticosteroid.
The vitamin D-naïve group comprised of 154 samples showed deficiency in 70%, insufficiency in 24%, and sufficiency in 6%.
The vitamin D supplemented group comprised of 463 samples was tested whilst on different maintenance or replenishment doses.
Three months replenishment regime of daily 3000 IU (N=23) or 6000 IU (N=37) achieved sufficiency in 52% and 84%, respectively.
22 samples were on 200 IU, with deficiency in 13(59%) and insufficiency in 7 (32%). 182 samples were on 400 IU, with deficiency in 104(57%) and insufficiency in (37%). 97 samples were on 800 IU, with deficiency in 19 (20%) and insufficiency in 56 (58%). 81 samples were on 1000 IU and 14 samples were on 1500 IU, with sufficiency in 40(50%) and 9(64%), respectively. No toxic level was seen in this cohort (highest level -230 nmol/l)
Conclusion:
The prevalence of Vit D deficiency/insufficiency in DMD is high. Replenishment regime of 6000 IU was more effective than 3000 IU in achieving Vit D sufficiency. Maintenance doses of 200 or 400 IU achieved sufficiency only in 14%. 1000 IU achieved sufficiency in 57%. These data have important implications for optimizing vitamin D dosing in DMD.

P32

Estrogen related receptor gamma gene transfer upregulates oxidative and angiogenic factors in *mdx* mice.

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Duchenne muscular dystrophy (DMD) is the most severe form of muscular dystrophy. DMD is an inherited X-linked neuromuscular disorder with an incidence of 1:4000 male births, caused by the absence of dystrophin. Loss of dystrophin causes progressive loss of muscle fibres which leads to cardiac and respiratory failure and ultimately death within the third decade. Pathological consequences of dystrophin deficiency include sarcolemmal instability, oxidative stress, calcium dysregulation, fibre necrosis, hypoxia and inflammation. Estrogen related receptor gamma (ERRγ) is an orphan nuclear receptor, exhibiting all the hallmarks of an oxidative and angiogenic master switch. Targeting signaling pathways, such as ERRγ, that is deregulated in DMD might help in ameliorating disease pathology and restore oxidative metabolism and angiogenesis in dystrophin deficient skeletal muscle. We have shown that *mdx* mice transgenic for ERRγ improves sarcolemmal integrity and promotes muscle perfusion, with the restoration of metabolic and angiogenic genes.
Adeno-associated virus serotype 8 (AAV8) expressing murine ERRγ (5×10^{10}vg) was administered by an intramuscular injection into Tibialis anterior (TA) muscle of either 6 week or 12 week old *mdx* male mice (n=6). Compared to control injected mice, TA muscle recovered 6 week post-administration did not demonstrate any significant increase in muscle mass, muscle diameter and muscle fibre diameter in any cohort; nor was there a difference in myosin heavy chain fibre typing. Succinate dehydrogenase analysis of Tibialis anterior muscles demonstrated a significant increase in oxidative muscle fibres in both cohorts (6 week cohort 31% to 67%, p=0.002; 6 week cohort 41% to 65%, p=0.001). Quantitative analyses of oxidative, metabolic and angiogenic transcripts are ongoing and will be presented.

P33

Nuclear protein spreading: implication for pathophysiology of neuromuscular diseases

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Background: While transfer of a protein encoded by a single nucleus to nearby nuclei in multinucleated cells has been known for almost 25 years, the biological consequences for gain-of-function diseases have not been considered.
Aims: We have investigated nuclear protein spreading and its potential consequences in two of the three most prevalent neuromuscular diseases.
Methods: We performed co-cultures between diseased or control human myoblasts and murine C2C12 myoblasts.
Results: We demonstrated that in facioscapulohumeral dystrophy (FSHD), although the transcription of the toxic protein DUX4 occurs in only a limited number of nuclei, the resulting protein diffuses into nearby nuclei within the myotubes, thus spreading aberrant gene expression. In myotonic dystrophy type 1 (DM1), we observed that in human-mouse heterokaryons, the expression of a mutated DMPK from human nuclei titrates splicing factors produced by neighboring nuclei, inducing the mis-splicing of several pre-mRNAs in murine nuclei.
Conclusion: In both FSHD and DM1, the spreading of the pathological phenotypes from one nucleus to another is observed, highlighting an additional mechanism that contributes to the dissemination and worsening of the muscle pathogenesis. These results indicate that nuclear protein spreading may be an important component of pathophysiology of gain of function muscular diseases which should be taken into consideration in the design of new therapeutic approaches.

Mitochondrial Disease

‡P34

The OPA1^{Q285STOP}/RedMIT/GFP-LC3 mouse to understand the implication of mitophagy in the autosomal dominant optic atrophy

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Background: Autosomal dominant optic atrophy (ADOA) is the most common inherited optic neuropathy (prevalence 1:12 000) resulting in a bilateral, symmetrical and painless loss of visual acuity, colour vision defects, central visual field loss and atrophy of the optic disc. It is a slowly progressive neuropathy, currently irreversible and untreatable. Over 200 mutations in the essential gene *OPA1* have been identified in patients with ADOA. It encodes a ubiquitous protein involved in the regulation of the mitochondrial network and cristae morphology, in the oxidative phosphorylation and membrane potential maintenance and in apoptosis. The tissue specificity is still not understood however the *OPA1*^{Q285STOP} mouse model shows increased levels of autophagy and some RGCs with aberrant cristae. These results combined with our observation that patient fibroblasts have increased mitophagy lead us to investigate the role of this mitochondria recycling process in ADOA.
Aims: Investigate mitophagy in an ADOA model.
Methods: We crossed the *OPA1*^{Q285STOP} mouse with our RedMIT/GFP-LC3 mouse, harbouring red fluorescent mitochondria and green fluorescent autophagosomes. We will monitor the co-localisation between mitochondria and autophagosomes in tissues, splenocytes and MEFs with Imagestream (Amnis), InCell analyser (GE) and confocal microscopy.
Previous results in the RedMIT/GFP-LC3 mouse confirmed that we could monitor it and detect an increase when the lysosomal processing was impaired.
Results: Preliminary results from an Imagestream experiment showed that the level of mitophagy is increased in splenocytes from the OPA1^{Q285STOP}/RedMIT/GFP-LC3 mice when compared to the RedMIT/GFP-LC3 control mice. RGCs from these mice are under investigation and MEFs will be produced to further study and confirm the implication of mitophagy in the development of the disease.
Conclusions: The preliminary results seem to confirm the increase in mitophagy found in patients. We will need to confirm it in RGCs and further investigate its impact on cell life.

‡P35

Quantification of mitochondrial respiratory chain function in single muscle fibres: important implications for diagnosis and treatment

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Background: Mitochondrial dysfunction is known to occur in mitochondrial myopathies, ageing and several neurodegenerative diseases. Patients with mitochondrial myopathies frequently show a mosaic pattern of mitochondrial dysfunction as demonstrated by the sequential cytochrome *c* oxidase /succinate dehydrogenase (COX/SDH) histochemical assay which is currently the standard method to assess mitochondrial respiratory chain function in individual cells and tissue sections. Conventional image analysis relies on visual examination making the assessment subjective and challenging. Furthermore, the available mitochondrial histochemical assays are not able to evaluate complex I activity, which is the commonest respiratory chain deficiency observed in children and adults with mitochondrial disease.
Aim: To develop a robust, objective and semi-automatic technique to quantify the degree of mitochondrial dysfunction in individual muscle fibres.
Method: Our novel method is based on quadruple immunofluorescence using antibodies detecting: Complex I (NDUFB8) and Complex IV (COX1) as well as porin (a marker of mitochondrial mass) and laminin (a cell membrane marker). Optical density (OD) of NDUFB8, COX1 and porin are simultaneously measured in single muscle fibres (IMARIS software) and used to classify fibres according to COX1 and NDUFB8 levels.
Results: This technique allows quantification of protein expression in single muscle fibres to provide an objective assessment of respiratory chain deficiency in muscle. We show that patients bearing different mitochondrial genetic defects –both mtDNA and nuclear mutations - have a different biochemical signature in muscle (with different proportions of COX1 and NDUFB8 deficiency) which is consistent among patients with identical genotypes.
Conclusion: This new tool will provide important insights into the nature of the mitochondrial biochemical defect seen in patients with different mitochondrial genetic defects and myopathies in which mitochondrial dysfunction is implicated. Furthermore, we believe that it will be useful in the rapid diagnosis of mitochondrial disease and as a validated outcome measure of mitochondrial function in clinical trials assessing the effectiveness of new treatments.

‡P36

Investigation of GARS associated mitochondrial dysfunction *in vitro* and *in vivo*.

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Mitochondrial disorder refers to diseases that are caused by OXPHOS dysfunction and they lead to clinically and genetically heterogeneous group of syndromes which are amongst the most common inherited human diseases. An increasing fraction of mitochondrial protein synthesis deficiencies are caused by mutations in mitochondrial aminoacyl tRNA synthethases (mt-ARSs) genes and causes combined RC deficiency showing tissue specific clinical presentations. mt-ARSs are a group of nDNA encoded mitochondrial proteins and they are required for normal mitochondrial protein synthesis by charging the tRNAs with their cognate amino acids at the beginning of the translational process. The *GARS* gene encodes both cytoplasmic and mitochondrial glycyl-tRNA-synthetase. Heterozygous *GARS* mutations were reported in autosomal dominant neuropathy with upper limb predominance and autosomal recessive mutations in patients with cardiac and skeletal muscle phenotype including mitochondrial abnormalities.
Our aim was to investigate how mutations within the *GARS* gene causing neuropathy affect mitochondrial function and whether an impaired mitochondrial function contributes to the disease.
We have investigated the downstream effect of siRNA mediated downregulation of GARS in human cell lines and we performed studies on a previously reported mouse model, the $GARS^{C201R}$ mouse.

We have found that *in vitro* ablation of GARS in human myoblasts predominantly disturbed the steady state levels and activity of complex I which resulted in abnormal mitochondrial protein synthesis. The $Gars^{C201R}$ mouse model (equivalent to human C157R mutation) was used to study the mitochondrial function of different tissues (brain, muscle, heart, liver, and kidney) and cells of the $GARS^{C201R/C201R}$ mice. Unexpectedly, no significant defect of mitochondrial protein synthesis was detected in any of the tissues in heterozygous and homozygous mice, although homozygous mice died before 17 days of age.
We suggest that some *GARS* mutations affect peripheral nerves only, while others cause mitochondrial disease, which reflects new insights into two independent disease mechanisms.

P37

Investigating mitochondrial dysfunction in myofibrillar myopathies.

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Introduction: Myofibrillar myopathies (MFM) are a group of myopathies characterised by focal myofibrillar destruction and accumulation of myofibrillar elements as protein aggregates. Previous research has implicated the myofibrillar destruction with changes in mitochondrial positioning, dynamics, mitochondrial DNA (mtDNA) rearrangements and evidence of focal respiratory chain deficiency.
Aims: Identify and characterise mitochondrial dysfunction in a cohort of myofibrillar myopathy patients and to attempt understand the link between the nuclear genetic defects causing MFM and the observed mitochondrial dysfunction.
Methods: Long Range PCR was used to look for the presence of mtDNA rearrangements in individual COX-deficient fibre lysates by amplifying a 16.2 kb region of the mitochondrial genome. This was completed for patients with genetically-confirmed Desminopathy (n=4), Zaspopathy (n=1) and Myotilinopathy (n=1). Immunofluorescent staining for mitochondrial OXPHOS subunits including NDUFB8 (Complex I), COX1 (Complex IV) and Porin (mitochondrial marker) was completed on cryosectioned muscle from patients with Desminopathy (n=8), Zaspopathy (n=1) and Myotilinopathy (n=1).
Results: Long Range PCR studies revealed a small proportion of the observed COX-deficient fibres harbour clonally-expanded mtDNA rearrangements. Immunofluorescent staining demonstrated a low percentage of COX-deficient and COX-intermediate fibres within the Desmin patients as well as a low level of Complex I-deficient and Complex I-intermediate fibres. More strikingly, higher levels of COX-deficient and COX-intermediate fibres were present in the patient with a ZASP mutation. The most prominent defect in of patients examined however was the proportion of fibres expressing low or very low levels of Porin, indicative of mitochondrial depletion.
Conclusion: MFM patients within our cohort appeared to have varying degrees of mitochondrial dysfunction. The decreased expression of mitochondrial Porin seems to be prominent and may result due to aggregation of mitochondria in the subsarcolemmal region. The low level of clonally-expanded mtDNA rearrangements observed are likely to be a consequence of age-related somatic mtDNA mutation.

P38

Improving the diagnosis of paediatric mitochondrial disease using high throughput sequencing technologies

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Background: Mitochondrial diseases affect both children and adults and are caused by defects in one of the ~1400 genes (only 13 of which are mtDNA-encoded) that encode the body's energy-transducing machinery. Often involving muscle and brain, they are clinically and genetically heterogeneous, therefore difficult to diagnose. Establishing a genetic diagnosis facilitates access to genetic counselling and prenatal genetic diagnosis. Isolated complex I deficiency is the most common biochemical defect in paediatric mitochondrial disease but current diagnostic technologies cannot determine the defect in 50% of patients.
Aim: To implement a targeted next-generation sequencing (NGS) strategy for paediatric patients with a clinical and biochemical diagnosis of isolated complex I deficiency. A rapid result is crucial in a diagnostic setting, particularly if it can assist reproductive choices for an ongoing pregnancy.
Methods: A custom Ampliseq panel that targets all 50 candidate genes and high-throughput sequencing using an IonTorrent PGM sequencer allows rapid and cost effective analysis. Bioinformatic assessment of variants allows prioritisation of candidate mutations and familial screening confirms segregation. Functional characterisation of novel mutations by western blotting, BN-PAGE and cDNA analysis were undertaken where appropriate to provide evidence of pathogenicity.
Results: A genetic diagnosis has been obtained for approximately 25% of this patient cohort (18/83 patients), facilitating familial carrier testing and access to reproductive counselling. Mutations in assembly factors are a common cause of isolated complex I deficiency with mutations in ACAD9, NDUFAF2*, NDUFAF5*, *NDUFAF6* and *FOXRED1* having been identified in our cohort, as have mutations in structural subunits including *NDUFS2*, *NDUFS4* and *NDUFS6*.
Conclusion: The integration of a targeted NGS methodology into our existing NHS laboratory service has provided a rapid genetic diagnosis for a number of patients. For unresolved cases, whole-exome or whole-genome sequencing may provide a genetic diagnosis. This strategy serves to deliver immediate patient benefit through the provision of genetic counselling to inform reproductive choices.

P39

Long-term survival in a child with severe encephalopathy, multiple respiratory chain deficiency and *GFM1* mutations

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Background: Mitochondrial diseases due to deficiencies in the mitochondrial oxidative phosphorylation system (OXPHOS) can be associated with mutations in the mitochondrial genome (mtDNA) or *nuclear* genes encoding

mitochondrial proteins. A subset of such nuclear mutations affect proteins involved in mitochondrial translation, causing heterogeneous, early-onset and often fatal clinical phenotypes.
Aims: To describe the clinical presentation and diagnostic work-up of an infant with multiple mitochondrial respiratory chain defects due to mutations in *GFM1*, which encodes the mitochondrial translation elongation factor G1 (mtEFG1).
Patient: Currently 5 years 6 months old, the female patient presented at the age of 2 months with severe encephalopathy, spastic-dystonic tetraparesis, failure to thrive, seizures and persistent lactic acidosis. Brain imaging revealed thinning of the corpus callosum and diffuse alteration of white matter signal.
Results: Biochemical assessment of respiratory chain enzymatic function showed impaired Complex I, III and IV in patient fibroblasts and histochemical analysis of muscle tissue showed a mosaic pattern of COX deficiency. Genetic investigation confirmed recessively inherited mutations in the *GFM1* gene, comprising a novel frameshift mutation (c.1404delA, p.(Gly469Valfs*84)) and a previously reported missense mutation c.2011C>T, p.(Arg671Cys). Mitochondrial protein synthesis and steady state protein levels of mtEFG1 were severely reduced in patient fibroblasts, confirming pathogenicity of the *GFM1* mutations.
Conclusions: Mutations in the *GFM1* gene confer high susceptibility to neurologic or hepatic dysfunction. Our patient shares many clinical, laboratory and radiological similarities with the eleven patients reported in the literature with mutations in this gene, but presents with a stable clinical course without metabolic decompensations, rather than a rapidly progressive fatal course, indicating that patients with defects in the *GFM1* gene can survive beyond early childhood.

P40

Reproductive options for women with mitochondrial DNA disease

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Pathogenic mutations in mitochondrial DNA (mtDNA) are highly prevalent in the population and can result in a range of isolated organ or multisystem disorders affecting children and adults, collectively known as mtDNA disease. The clinical features of these diseases are extremely variable, ranging from no symptoms or very mild symptoms to severely debilitating disease or death in early infancy. The mitochondrial genome is strictly maternally inherited and so a woman with an mtDNA mutation is at significant risk of passing this to her children through the mitochondria present in her oocytes. The risk is very difficult to predict, however, due to a genetic bottleneck that occurs during development of the female germline. This uncertainty, coupled with the lack of an effective treatment, means that women with mtDNA disease have difficult decisions to make about having children.
The current reproductive options available to women with an mtDNA mutation will not be suitable for all. To address this problem, we have developed a novel IVF-based technique to prevent transmission of mtDNA disease. The technique, termed mitochondrial replacement, involves transferring the nuclear DNA from the egg of an affected woman to a donated egg from which the nuclear DNA has been removed. The resulting reconstituted egg would enable the woman to have a child that is genetically related to both parents but with a greatly reduced risk of mtDNA disease.
Experiments to determine the safety and efficacy of mitochondrial replacement have been performed and are crucial if this technique is to be considered as a clinical treatment to prevent transmission of mtDNA disease. The use of mitochondrial replacement has ethical and legal implications and requires a change in policy. This has been an extensive process and the proposed technique is to be debated in both Houses of Parliament in early 2015.

P41

Mitophagy and mitochondrial morphology in patients with the m.13051G>A mitochondrial DNA mutation

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Background: Mitochondria have diverse functions within the cell, from supplying cellular energy, to signalling, cellular differentiation and cell death. Mitochondrial diseases that result from maternally inherited mitochondrial DNA (mtDNA) mutations occur in 1/400 individuals. Leber's Hereditary Optic Neuropathy is one such disease in which, predominantly, young males lose vision acutely.
Mitophagy is a cellular mechanism for the recycling of redundant or dysfunctional mitochondria. Defects in mitochondrial quality control, dysregulated mitophagy and mitochondrial dynamics have been related to an array of neurological disorders.
Aims: Quantify mitophagy and investigating mitochondrial morphology in cultured primary fibroblasts derived from members of a family harbouring the m.13051G>A pathogenic mitochondrial DNA mutation associated with both Leber's Hereditary Optic Neuropathy and Leigh's disease.
Methods: We used a previously developed high throughput imaging method for quantifying mitophagy and investigating mitochondrial morphology in cultured primary fibroblasts derived from four affected members of two families harbouring the homoplasmic m.13051G>A mitochondrial genetic mutation. Patient derived fibroblasts were cultured either in standard 25mM glucose media or galactose, glucose free, media. Cells were immunostained for the autophagy marker LC3 and the mitochondrial protein TOM20 and analysed with INCell1000.
Results: We established that all patient cells have elevated levels of mitophagy along with a fragmented mitochondrial network when compared to healthy controls. Mitochondrial volume and mitochondrial DNA copy number was also increased in patient fibroblasts. Forcing the cells to use OXPHOS as an energy supplier, in galactose media, further increased the levels of mitophagy as well as mitochondrial length without decreasing mitochondrial volume.
Treatment of patient cells with idebenone decreased levels of mitophagy and also improved cell growth in vivo.
Conclusions: In conclusion, we provide evidence of increased mitophagy in LHON cells. We show that idebenone benefits cell growth and attenuates increased mitophagy. Drug modulators of mitophagy are potentially useful treatments for mitochondrial patients.

P42

***LRPPRC* mutations cause early-onset multisystem mitochondrial disease and COX deficiency outside of the French Canadian population**

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Background: Mitochondrial complex IV (cytochrome *c* oxidase (COX)) deficiency is one of the most common respiratory chain defects in humans. The clinical phenotypes associated with COX deficiency include liver malfunction, cardiomyopathy and Leigh syndrome, a neurodegenerative disorder associated with mutations in several genes. A founder mutation in the *LRPPRC* gene encoding the leucine-rich pentatricopeptide repeat domain protein (LRPPRC) - involved in post-transcriptional regulation of mitochondrial gene expression - causes the French Canadian variant of COX-deficient Leigh syndrome (LSFC) unique to the Saguenay-Lac-Saint-Jean region of Quebec.
Aim: To characterise the clinical and molecular nature of novel *LRPPRC* mutations identified in patients with early-onset COX deficiency associated with multi-system involvement.
Patients and Methods: Using whole exome and candidate gene sequencing we identified eight patients from five unrelated families of UK-Caucasian, UK-Pakistani and Turkish origin harbouring biallelic mutations in the *LRPPRC* gene. Patients manifested a wide range of clinical features including neonatal-onset lactic acidosis, hypoglycaemia, hypotonia, neurodevelopmental delay and cardiomyopathy and all were diagnosed with isolated COX deficiency in muscle.
Results: Functional characterisation of LRPPRC patients' fibroblasts and skeletal muscle homogenates showed decreased levels of mutant LRPPRC protein and impaired complex IV enzyme activity. This was associated with abnormal COX assembly, and reduced steady state levels of numerous OXPHOS subunits. Moreover, we identified a Complex I assembly defect in skeletal muscle, indicating different roles for LRPPRC in post-transcriptional regulation of mitochondrial mRNAs between tissues. Although patient fibroblasts showed decreased steady state levels of mitochondrial mRNAs, the length of the poly(A) tails were unaffected.
Conclusion: Our study identifies *LRPPRC* as an important pathogenic gene causing multi-organ early-onset mitochondrial disease associated with variable OXPHOS defects and should be considered as a cause of COX deficiency even in patients originating outside of the French Canadian population.

P43

Whole exome sequencing identifies mutations in the CLPB protein disaggregase as a novel cause of mitochondrial disease with 3-methylglutaconic aciduria

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Background: 3-Methylglutaconic aciduria (3MGA) is a feature of clinically and genetically heterogeneous disorders of mitochondrial metabolism, including Barth syndrome (X-linked dilated cardiomyopathy and cyclical neutropaenia caused by tafazzin deficiency), Sengers syndrome (cataracts and cardiomyopathy caused by acylglycerol kinase deficiency) and MEGDEL syndrome (Leigh-like disease caused by SERAC1 deficiency).

Aims: We sought to determine the genetic basis of 3MGA in two siblings with an unusual phenotype of congenital lamellar cataracts and nephrocalcinosis with renal medullary cysts.
Methods: Whole exome next generation sequencing was performed in two siblings born to healthy unrelated parents. Filtering and bioinformatics analysis assumed autosomal recessive inheritance in a gene encoding a mitochondrially targeted protein. Potentially pathogenic mutations were confirmed by Sanger sequencing, and functional analysis included quantitative PCR (qPCR), Western blotting and molecular modelling.
Results: The only bi-allelic variants identified in a gene encoding a mitochondrially targeted protein were c.1882C>T (p.Arg628Cys) and c.1915G>A (p.Glu639Lys) in the *CLPB* gene. *CLPB* encodes caseinolytic peptidase B homologue, a heat shock protein/chaperonin responsible for disaggregating mitochondrial and cytosolic proteins. Western blot analysis revealed reduced steady state levels of CLPB protein in cultured patient fibroblasts, supporting pathogenicity of these mutations. Molecular modelling suggested that the mutations disrupt interactions between subunits so that the CLPB hexamer cannot form or is unstable, thus impairing its role as a protein disaggregase.
Conclusion: We conclude that accumulation of protein aggregates underlies the development of cataracts and nephrocalcinosis in CLPB deficiency, which is a novel genetic cause of 3MGA. A common mitochondrial cause for 3MGA appears to be disruption of the architecture of the mitochondrial membranes, as in Barth, Sengers and MEGDEL syndromes (all characterised by impaired biosynthesis or remodelling of the mitochondrial membrane lipids). We now propose that perturbation of the mitochondrial membranes by abnormal protein aggregates leads to 3MGA in CLPB deficiency.

P44

Investigation of an intronic OPA1 mutation causing optic atrophy, coupled with an intralocus modifier linked to additional extra-ocular neuromuscular disease

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Background: Inherited optic neuropathies are early onset mitochondriopathies characterised by retinal ganglion cell degeneration and subsequent bilateral vision deficiency. The nuclear gene OPA1 encodes the dynamin-like 120kDa protein, integral in processes such as apoptosis regulation and mitochondrial fusion, and it has been linked to dominant optic atrophy in particular. Several exonic missense mutations in this gene are currently known, but a recent study on previously unsolved cases of optic atrophy unveiled two deep intronic causative mutations in OPA1, the first time this type of mutation has been associated with inherited optic neuropathies. Additionally, cases compound heterozygous for a specific known exonic SNP were strongly correlated with presenting with a syndromic phenotype of additional ataxia and neuromuscular disease.
Aims: We predict that these two deep intronic mutations could underlie several more undiagnosed optic atrophy cases and, when coupled with the exonic variant, could underlie complex optic atrophy cases with a further ataxic/neuromuscular disease phenotype.
Methods: To investigate these mutations, we will sequence the DNA of 217 patients with unexplained optic atrophy. The majority of these patients had presented with additional extra-ocular symptoms such as deafness, ataxia or neuromuscular disease.
Results: Results have yet to be obtained, but it is expected that further instances of these deep intronic mutations underlying cases of optic atrophy will be identified. Furthermore, any cases compound heterozygous with the exonic variant will be linked to syndromic optic atrophy.

P45

Novel alanyl-tRNA synthetase 2 (*AARS2*) mutations causing fatal infantile cardiomyopathy and respiratory failure

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Background: Multiple mitochondrial respiratory chain complex deficiencies due to mutations of nuclear-mitochondrial genes present vast clinical heterogeneity in patients. Given the large number of genes, whole exome sequencing (WES) represents an effective tool for identifying and prioritising likely causal variants.
Aims: To identify and prioritise variants in nuclear genes encoding mitochondrial proteins in a patient presenting with multiple mitochondrial respiratory chain complex deficiencies and infantile cardiomyopathy.
Patient and Methods: The proband was a boy born to non-consanguineous parents who presented with severe myopathy, metabolic acidosis, and respiratory insufficiency requiring non-invasive ventilation from birth and died at seven weeks of age. Cardiomyopathy was initially absent but developed by five weeks of age, manifesting as mild biventricular hypertrophy and a significant conduction defect. Biochemical studies revealed severe complex I, III, and IV deficiencies while muscle histochemistry showed vacuolated fibres with increased lipid and >50% COX-deficient fibres. Sequencing of mtDNA was normal. Family history was significant; an older sibling died at 24 hours from severe lactic acidemia, pulmonary hypoplasia, and coagulopathy.
Results: We identified two novel heterozygous (p.(Asp337*) and p.(Arg580Trp)) *AARS2* variants. Studies to confirm pathogenicity include western blot analysis of OXPHOS and AARS2 protein, and modelling the effect of these variants on the AARS2 structure.
Conclusion: To our knowledge, this is the first patient with *AARS2* mutations and infantile cardiomyopathy not to carry the founder p.(Arg592Trp) mutation. Structurally, both p.(Arg580Trp) and p.(Arg592Trp) mutations occur in the functionally conserved editing domain of AARS2, suggesting a similar pathogenesis leading to infantile cardiomyopathy. We propose that mutational screening of *AARS2* should be considered in patients presenting with multiple mitochondrial respiratory chain complex deficiencies and infantile cardiomyopathy, irrespective of onset.

P46

A commonly used treatment for mitochondrial diabetes inhibits mitophagy in heteroplasmic patient cultures under mitochondrial energetic stress

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Background: The m.3243A>G mutation is one of the commonest mtDNA mutations, causing oligosymptomatic presbycusis or diabetes rather than mitochondrial myopathy in most cases. Mutant mtDNA co-exists with wild type (heteroplasmy), with the mutant load determining severity. Mitophagy (recycling of damaged mitochondria)

is likely to be an important determinant of disease progression in such heteroplasmic mtDNA disease. However documenting mitophagy is technically demanding.
Aims: To determine whether mitophagy underlies the drop in load of mutant mtDNA seen during m.3243A>G cultures under mitochondrial energetic stress.
Methods: We validated high throughput fluorescence microscopy for detecting mitophagy in m.3243A>G and control fibroblasts.
Results: By monitoring load of heteroplasmic mutant mtDNA, mtDNA copy number and mitochondrial mass, we show that mitochondrial energetic stress reduces mutant mtDNA in fibroblasts. Mitophagy is implicated because it is accompanied by increased LC3 puncta containing mitochondria, (the hallmark mitophagy) and the mutant load correlates inversely with the level of LC3-II (p=0.05). Co-localisation of LC3 puncta with mitochondria was significantly increased in heteroplasmic cells compared to wild type under energetic stress caused by galactose but not at baseline. Inhibiting mitophagy either genetically or with drugs reduced co-localisation of LC3 with mitochondria.
We conclude that Mitophagy removes pathogenic mutant mtDNA from heteroplasmic patient cultures under mitochondrial energetic stress.
We used this system to screen for the effects of various drugs on mitophagy. We found that a commonly used oral hypoglycaemic agent impaired mitophagy. This could have long-term effects on accumulation of mutant mtDNA by heteroplasmic patients. Furthermore it could be particularly damaging under mitochondrial energetic stress such as fasting.
Conclusion: We suggest that inhibitors of mitophagy are not ideal therapies for patients with heteroplasmic mtDNA disease. We recommend screening drugs to identify those that modulate mitophagy. In the case of maternally inherited diabetes and deafness due to m.3243A>G, insulin has some advantages.

P47

Reproductive decision making in mitochondrial patients: a qualitative investigation of women's experience

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Background: Mitochondrial disease is a group of disorders that are heterogeneous and are caused by defects in the mitochondrial DNA (mDNA) or in the nuclear DNA of a cell that affects mitochondrial maintenance. They are a clinically and genetically diverse group of disorders that are progressive and disabling. There are many issues that make determining the risk of maternally inherited disease difficult. With newly available and emerging reproductive techniques, mitochondrial patients now have even more reproductive options than ever before to consider. There is currently no published research exploring how mitochondrial patients make reproductive decisions. Research into other genetic conditions does however give us a base to explore potentially similar factors affecting reproductive decision making in women with mitochondrial disease. Research into Huntington's disease for example has shown that lived/factual awareness of disease; parenting/child centred risk; personal values; concepts of future; risk mitigation and perceived social support impacted parental decision making. Studies into Duchenne Muscular Dystrophies have highlighted the importance of parental understanding of carrier and recurrence risk and that socio educational status can impact on this understanding.
Aims: We are interviewing up to 15 women in a retrospective group (retrospective accounts of decision-making) and up to 15 women who are currently or in the near future thinking about becoming pregnant (current and prospective accounts of decision-making).
Methods: Transcripts from semi structured interviews are being analysed using procedures from first-generation grounded theory, from analytic induction and constructionist grounded theory.
Conclusion: Preliminary analysis of interviews has shown that there are multiple factors that may influence decision making including 1) Impact of Diagnosis 2) Disclosure 3) Clinical Relationships and 4)Information Needs.

We hope the continued collection of data will allow for further insight into experiences of these women and the development of patient pathway to support reproductive discussions with mitochondrial patients.

P48

PRDM9 has zinc fingers in many pies

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Mitochondrial DNA deletions are present in many mitochondrial disorders as well as several age related diseases. Currently, 805 mtDNA deletions have been described however it is possible that others have not yet been observed due to low frequency or poor sensitivity of current techniques used to identify them. Many of these deletions are flanked by short repeat regions of DNA which may suggest specific regions of the mtDNA molecule have a susceptibility to deletion formation. PRDM9 is a meiotic-specific protein which is responsible for determining where recombination hotspots will occur in the nuclear genome. It binds to a specific DNA consensus sequence through its zinc finger region.
The aim of this study is to determine whether *PRDM9* plays a role in mtDNA deletion formation.
Grep and *Perl* scripts were used to screen mtDNA sequences from 9769 individuals for the presence of the *PRDM9* recognition motif. Genotyping of the *PRDM9* zinc finger repeat region was performed via Sanger sequencing in a cohort of 48 single deletion patients and 50 healthy controls. Immunodetection of PRDM9 was carried out using HEK-293 cell lysate. Subcellular fractions were achieved by differential centrifugation methods followed by SDS-PAGE and immunoblotting with anti-PRDM9 antibody. PRDM9 was immunoprecipitated using anti-PRDM9 antibody conjugated to A/G agarose beads.
The *PRDM9* recognition motif was found in 9760 of the sequences searched at a position in the mtDNA known to flank a previously described deletion. Interestingly, several sequences contained the motif more than once with some sequences containing up to 4 motif sites in total. Genotyping data showed no correlation between *PRDM9* allele status and the presence of the 'common' 4977bp mtDNA deletion in the cohort versus controls. Western blots of subcellular fractions showed PRDM9 protein is enriched in mitochondria of HEK-293 cells.
The *PRDM9* recognition motif previously described is present in mtDNA. PRDM9 protein appears to be enriched in the mitochondrial fraction of HEK-293 cells suggesting that PRDM9 may play a role in mediating mtDNA maintenance during development.

P49

Growth and differentiation factor 15 and fibroblast growth factor 21 are comparably sensitive and specific biomarkers of mitochondrial diseases

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Background: Growth and Differentiation factor 15 (GDF-15) is a cytokine of the transforming growth factor beta family regulated by p53 and oxidative stress. We recently described increased levels of GDF-15 in muscle and

serum from patients with mitochondrial disease suggesting that it could represent a novel biomarker for this group of complex disorders.
Aims: To evaluate the use of GDF-15 in the diagnosis of mitochondrial diseases relative to Fibroblast-Growth-Factor 21 (FGF-21) and other biochemical parameters.
Methods: Circulating GDF-15 and FGF-21 were measured in serum or plasma by ELISA. Other metabolites were determined using standard procedures. Statistical analysis was performed using SPSS v.20.
Results: We have studied 22 samples from 19 patients with confirmed mutations in nuclear (n=9) or mitochondrial (n=10) DNA, 23 samples from 20 patients with a definitive diagnosis of mitochondrial disease and 16 samples from 14 patients with a probable diagnosis of mitochondrial disease according to Morava criteria. We found that GDF-15 and FGF-21 were significantly ($p < 0.001$) increased in patients (both as a whole and for each sub-group) relative to controls. There was a significant correlation between GDF-15 and FGF-21 levels and between both factors and Asparte and Alanine aminostransferases. ROC analysis was performed to evaluate the sensitivity and specificity of each factor for different cut-off values. The area under the ROC curve was 0.80 for GDF-15 and 0.78 indicating that both tests have a good discriminatory power.
Conclusion: Diagnosis of mitochondrial diseases is challenging, particularly in children, and the availability of specific and sensitive serum biomarkers would be very helpful to select patients for more complex biochemical and genetic analysis. Our data show that GDF-15 is a valuable marker for such purposes and that it is as sensitive and specific as FGF-21. We propose to include both factors in the diagnostic workup of these diseases.

P50

Studying the effect of L-cysteine in mitochondrial disease

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Background: Mitochondrial disorders comprise a large group of heterogeneous disorders characterized by impaired cellular energy production. Mutations located within the mt-tRNA genes are a common cause of mitochondrial disorders.
We have previously reported that L-cysteine prevented the decrease of mitochondrial translation in cells of patients with reversible infantile respiratory chain deficiency and TRMU deficiency, supporting the hypothesis that low cysteine concentrations may play a role in triggering a reversible *in vitro* mitochondrial translation defect.
Aim and Methods: Based on these observations we expanded the supplementation with L-cysteine to other mitochondrial conditions affecting posttranscriptional modifications of mt-tRNAs on the molecular level. Absence of post-transcriptional modifications at the wobble positions of mitochondrial tRNAs for Leu and Lys has been correlated to Mitochondrial Encephalomyopathy and Lactic Acidosis with Stroke-like episodes (MELAS) and Myoclonic Epilepsy with Red Ragged Fibres (MERRF) syndromes, respectively. We tested whether supplementation of growth media with L-cysteine can reverse the defect in cells from patients with MELAS and MERRF, as well as in patients with other types of mitochondrial translation defects (COX10, MTO1 and ELAC2 mutations). Mitochondrial complex assembly was assessed by BN-PAGE, in-gel activity assay, and oxygen consumption (Seahorse analysis).
Results and conclusions: Our data indicate increased levels of mitochondrial complexes after L-cysteine supplementation in some, but not all of the studied patient cell lines. We are testing whether our approach of supplementation with L-cysteine could be used in the future to treat some mitochondrial conditions.

P51

Why do patients with mitochondrial disease get worse over time: An investigation into genetic and cellular mechanisms of disease progression

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Background: Mitochondrial diseases are among the most prevalent genetic disorders worldwide, affecting approximately 1/5000 people. Little is known about disease progression, but understanding mechanisms may guide drug development and treatment strategies. Previous studies have suggested that mitochondrial DNA (mtDNA) mutation load (heteroplasmy) and mtDNA copy number may be associated with disease severity and progression. However, evidence for particular aspects of these hypotheses is sparse and often conflicting. Increases in mtDNA heteroplasmy level and mtDNA copy number can indicate respiratory (biochemical) dysfunction of mitochondria and so detecting changes in the levels of respiratory chain protein expression is also important.
Aims: This longitudinal study aims to investigate the molecular genetic and cellular changes in serial skeletal muscle biopsies from patients with either the m.3243A>G point mutation (n=6) or single, large-scale mtDNA deletions (n=10).
Methods: Real-time PCR based assays were used to determine mtDNA copy number in muscle biopsy homogenates. Muscle heteroplasmy levels were determined using quantitative pyrosequencing (m.3243A>G mutation) or real-time PCR (single, large-scale deletions). Immunofluorescence was performed on serial muscle biopsy sections to interrogate immunoreactivity for COXI (complex IV subunit), NDUFB8 (complex I subunit) and porin as a marker for mitochondrial mass. Clinical progression was measured using a validated NMDAS (Newcastle Mitochondrial Disease Adult Scale) rating scale.
Results: mtDNA heteroplasmy and mtDNA copy number were shown to change over time in patient muscle with m.3243A>G and single large-scale mtDNA deletion mutations, however no consistent correlations were identified across the cohort. Quantitative densitometric analysis of COXI, NDUFB8 and porin levels also indicated differences in the abundance of these proteins between serial muscle biopsies.
Conclusion: Molecular genetic changes do not appear to be immediately associated with clinical progression scores, however a deeper analysis of the changes in expression of mitochondrial OXPHOS subunits may reveal a relationship between genetic, cellular and clinical disease progression.

P52

Gait analysis in patients with mitochondrial disease

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Background Mitochondrial diseases are a heterogeneous group of genetic disorders, resulting in numerous symptoms that affect walking. Selective gait characteristics have been shown to be sensitive to mitochondrial pathology, disease burden and genotype. In other conditions more expansive gait analysis has revealed further gait deficits and cognitive limitations and may be of use in mitochondrial disorders.
Aims Evaluate how gait in patients with mitochondrial disease may be affected by the addition of a secondary task whilst walking and the potential impact of fatigue on gait.
Methods Twenty-four patients with mitochondrial disease (m.3243A>G, m.8344A>G) and 24 healthy controls were recruited. Gait analysis was performed using an instrumented walkway (GAITRite). Patients performed single, dual and continuous walks.

Results Compared to controls, patients with mitochondrial disease were globally impaired in all characteristics of gait. Patients thought to be asymptomatic carriers of the m.3243A>G mutation, revealed differences in a discreet number of gait characteristics (gait speed, step length, variability in step time and step width) compared to controls. No further gait characteristics were revealed to be different with the addition of a dual task and no dual task interference was noted. This was repeated with prolonged walking with no deterioration in gait characteristics over the course of the walk.
Conclusion The addition of a secondary task whilst walking and the performance of a prolonged walk were unable to reveal any latent motor deficits in this group of high functioning mitochondrial patients. Gait characteristics were also unable to detect fatigue over a prolonged walk.

P53

Gait analysis: accuracy as a clinician reported measure in mitochondrial disease.

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Background A recent Cochrane review highlighted the difficulties in fully evaluating clinical trials investigating treatments of mitochondrial myopathy due to the lack of consistent outcome measures applied across all studies.This is due in part to the extreme variability in clinical phenotypes and the lack of reliable clinical evaluation tools to assess disease progression.
Aims To preliminary assess the accuracy of physiological, functional and gait measures in patients with mitochondrial disease.
Methods Twenty-four patients with mitochondrial disease (m.3243A>G, m.8344A>G) and 12 controls underwent clinical assessment including exercise testing, isokinetic dynamometry, functional measures (5-times-sit-to-stand (5XSTS), (Timed-up-and-Go (TUG), 10m-timed walk (10MTW), 6 minute walk (6MWD)) and gait analysis. Receiver Operating Characteristic (ROC) Curves were calculated for each measure.
Results All measures were able to discriminate between the group of mitochondrial patients and control subjects ($p<.05$) at different levels of accuracy, with the most robust measure being the 5XSTS (Accuracy 89%, Sensitivity 92%, Specificity 83%, Area under the curve 0.934, $p<.001$). Whereas gait characteristics reported superior discrimination between genotypes, with step length variability being the most accurate (accuracy 96%, sensitivity 100%, specificity 83%, AUC 0.963, $p=.001$).
Conclusion Validation of clinician reported outcomes are essential prior to implementation in clinical trials within mitochondrial disease. Properties such as sensitivity and specificity should be considered alongside validity and reproducibility when choosing measures. Moreover, our data suggest that gait analysis may offer more sensitive and specific measures and should be considered as a robust, relevant clinician reported outcome in future clinical trials.

P54

Cardiac involvement in adult mitochondrial disease: the need for a genotype-specific management guideline

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Background: Cardiac involvement is a common clinical feature in mitochondrial disease, which can occur in isolation but more frequently as part of a multi-system disease. Similar to other neuromuscular diseases, cardiac dysfunction is the leading cause of death in mitochondrial disease. However, the natural history of cardiac involvement in different genotypes is poorly understood and so a uniform cardiac surveillance is generally adopted. It is uncertain whether asymptomatic mutation carriers benefit from such surveillance.
Aims: To identify frequency of cardiac involvement in common adult mitochondrial diseases (current age>=18 years) and adapt current mitochondrial disease management guidelines accordingly.
Methods: Clinical information on age, genotype, disease-burden measured using the Newcastle Mitochondrial Disease Adult Scale (NMDAS), serial ECGs and echocardiograms were retrieved from the MRC Mitochondrial Disease Patient Cohort database. Cardiac involvement was defined as having, at the very minimum, left ventricular hypertrophy or a non-sustained rhythm disturbance equating to a score of 2in question 9, section 2 of NMDAS. Descriptive statistical analysis was performed.
Results: Of 553 living patients in the Newcastle cohort the most common genotype is m.3243A>G (n=200) followed by single deletion (n=95), *PEO1* (n=39), m.8344A>G (n=37), *POLG1* (n=30) and others. Of these patients, 66% (n=365) have at least one NMDAS score recorded and 16% (n=63) have cardiac involvement. Cardiac involvement varies among the genotypes as shown here: m.3243A>G (n=30), single deletion (n=11), m.8344A>G (n=8), *PEO1* (n=2), *OPA1* (n=2) and *POLG1* (n=0). We have identified three sudden cardiac deaths (SCDs) in patients with the m.3243A>G mutation, two of whom were essentially asymptomatic.
Conclusion: Our results demonstrate that cardiac involvement is genotype specific and this would justify a more targeted cardiac management guideline. In view of the prevalence in population and increased recognition of SCDs in m.3243A>G, we propose comprehensive cardiac risk stratification in these patients and active family pedigree screening.

P55

A case of hepatocerebral mitochondrial DNA depletion syndrome caused by two novel splicing mutations in the *DGUOK* gene

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DGUOK (deoxyguanosine kinase) is one of several genes linked to disorders of mitochondrial DNA (mtDNA) maintenance. Mutations in *DGUOK* are associated with autosomal recessive hepatocerebral mtDNA depletion syndrome and with autosomal recessive progressive external ophthalmoplegia with multiple mtDNA deletions. In a cohort of patients with a suspected disorder of mtDNA maintenance, we identified an 11 year old boy with mtDNA depletion in liver (10% of normal mtDNA copy number), an atypical presentation, and two novel variants in the *DGUOK* gene, c.444-3C>G in intron 3 and c.592-26A>G in intron 4. Compound heterozygosity was confirmed by testing the unaffected parents. Although not affecting the critical last 2 nucleotides of an intron, c.444-3C>G was predicted to be pathogenic as a G nucleotide is rarely, if at all, found in the -3 position. *In silico* analysis suggested that c.592-26A may constitute the invariant A nucleotide of the intron 4 splicing branch point, and hence c.592-26A>G may lead to aberrant splicing. Analysis of fibroblast RNA by reverse transcription-PCR across the regions of interest showed that c.444-3C>G causes skipping of exon 4 in the majority of mRNA and c.592-26A>G causes skipping of exon 5 in the majority of mRNA. Both mutations result in frameshifts which are predicted to result in premature termination of translation and/or nonsense-mediated mRNA decay.
Thus, these results indicate that this patient's hepatocerebral mtDNA depletion syndrome was caused by insufficiency of full length normally spliced *DGUOK* mRNA due to compound heterozygous splicing mutations.

P56

Whole human mitochondrial genome next generation sequencing

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The human mitochondrial genome is a 16.6kb circular molecule present in 100s-1000s of copies per cell. Pathogenic point mutations are associated with primary mitochondrial DNA (mtDNA) disorders, and accumulation of point mutations can also be important in the pathogenesis of secondary mtDNA disorders. Historically, point mutations analysed by specific tests for known recurrent mutations and by Sanger sequencing of the genome. We have employed next generation sequencing technologies to sequence the mitochondrial genome with a high read depth (average coverage 5000 reads), and developed a bioinformatics pipeline to process the raw sequence data, enabling accurate and quantitative detection of point mutations at low levels of heteroplasmy.

Validation of our sequencing and bioinformatics pipeline was performed by comparing results for 12 diagnostic samples with those obtained by Sanger sequencing and similar NGS approaches in two other centres.

This assay can now be used to improve detection of primary mtDNA disorders, in some cases removing the need for muscle biopsy. In addition, we are developing this approach to investigate the presence of heteroplasmic point mutations in cell lines from patients with defects in mtDNA maintenance and/or mitochondrial quality control.

P57

Mitophagy deficiencies in mitochondrial DNA disease

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Mitophagy is the process of selective mitochondrial degradation that occurs to maintain efficient synthesis of ATP in the cell and avoids the toxic accumulation of damaged mitochondria. Dysfunction of mitochondria is linked to several neuromuscular and neurodegenerative disorders, due to the high energy requirements of skeletal muscle and the central nervous system. Within mitochondrial disease, mutated mitochondrial DNA (mtDNA) coexists with wild-type mtDNA in separate nucleoids and the severity of disease depends upon this ratio; therefore maintaining mitochondrial quality control processes is essential.

It has previously been shown that overexpression of Parkin, an E3 ubiquitin ligase recruited in mitophagy, can increase the ratio of wild-type mtDNA to mutant mtDNA in human cells (Suen *et al.,* 2010). Furthermore, it has also been suggested that mtDNA damage can induce mitophagy (Youle & Narendra, 2011).

The aims of this study are to assess the effect of mitophagy-inducing compounds, such as rhodamine-6G, on mtDNA, and to identify whether patients harboring mtDNA mutations show deficiencies in mitophagy. The effect of rhodamine-6G will be studied in a human neuroblastoma cell line (SH-SY5Y cells) and fibroblasts, by Western blotting, immunocytochemistry and next generation sequencing (NGS). Mitophagy in mitochondrial DNA disease will be studied using fibroblasts derived from patients presenting with a range of phenotypes, such as MERFF, MELAS, Leigh syndrome and ataxia. In addition, mitochondrial physiology will be characterised by live cell microscopy, to study ATP production and mitochondrial membrane potential, using tetramethylrhodamine methyl ester (TMRM) fluorescent probe.

P58

Chronic progressive external ophthalmoplegia – molecular genetic features and neurological burden

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Background: Chronic progressive external ophthalmoplegia (CPEO) is a classical manifestation of mitochondrial disease characterised by slowly progressive limitation of eye movements and ptosis. CPEO can develop either in isolation or as the defining feature of a more severe multisystemic "CPEO plus" phenotype.
Aims: As part of an epidemiological study of mitochondrial diseases in the UK, we set out: (i) to derive a robust estimate of the prevalence of CPEO in the general population; (ii) to define the molecular spectrum of disease causing genes associated with CPEO; and (iii) to document the overall burden of neurological disease in this group of patients.
Methods: Patients with suspected CPEO were identified from the Mitochondrial Disease Patient Cohort Study database and their medical records were then reviewed by two independent investigators (PYWM and AC) to confirm the diagnosis.
Results: Out of 631 patients that have been assessed by the Newcastle mitochondrial diagnostic service (June 2013), 255 patients (40.4%) were confirmed to have CPEO. The majority of patients (181/255, 71.0%) had typical ocular features of CPEO and the age of onset ranged from 1.0 to 76.0 years with a mean age of 29.9 years. Significant neurological deficits were present in 221 patients (86.7%) in particular myopathy (62.0%) and ataxia (51.0%). The minimum prevalence of CPEO was estimated at 3.39 per 100 000 inhabitants, about 1 in 30,000 of the general population. The most commonly identified genetic defect was a pathogenic mitochondrial DNA (mtDNA) point mutation (n = 80, 31.4%), followed by a single mtDNA deletion (n = 72, 28.2%), and a point mutation within nuclear-encoded CPEO genes (n = 70, 27.5%).
Conclusion: CPEO is a frequent cause of significant visual impairment among patients with confirmed mitochondrial disease and it is frequently associated with the development of debilitating neurological complications.

MRI

‡P59

Temporal profile of T2 MRI and 1H-MRS in the MDX mouse model of Duchenne muscular dystrophy

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Background*:* Duchenne muscular dystrophy (DMD) is an X-linked, lethal muscle wasting disease for which there is currently no treatment that effectively prevents the muscle necrosis and progressive muscle loss. The MDX mutant mouse model has been extensively studied as a model for DMD but to-date an extensive temporal, non-invasive profile that utilizes magnetic resonance imaging (MRI) and 1H-magnetic resonance spectroscopy (1H-MRS) has not been performed longitudinally in larger cohorts of rodents. In addition, longitudinal imaging has not coincided with attempts to exacerbate muscle damage by chronic exercise.

Aims: In this study we employed an 11.7 T small animal MRI in order to characterize the MRI and MRS profile of MDX mice longitudinally during a 9 month period during which MDX mice and WT were subjected to chronic exercise and then compared to MDX and WT mice that were not exercised.
Materials and Methods*:* Male MDX mice and male wild-type mice were subjected to a regime of treadmill running (20 min/session at14 meters/min) bi-weekly for 9 months. Gastrocnemius and tibialis anterior muscles were profiled with baseline T2-MRI and 1H-MRS at 6 weeks of age. Imaging and spectroscopy were repeated again at 3, 6 and 9 months. Plasma CK measurements coincided with imaging time-points.
Results: Results indicate that chronic exercise extends the dystrophic phenotype of MDX mice as evidenced by T2-MRI and1H-MRS when compared to MDX mice that were not subjected to chronic exercise..T2-MRI revealed the extent and location of the damage in gastrocnemius and tibialis anterior muscles as hyperintensities (lesions and edema) in exercised MDX mice. The magnitude of the muscle damage remained stable over time in exercised mice while it decreased to near WT levels in the unexercised mice. Very little evidence of fat infiltration or
accumulation in the muscle tissue was seen at any time-point in both MDX groups. Creatine, choline and taurine levels evaluated by 1H-MRS
were found to be significantly decreased at each time-point. Extramyocellular lipid (EMCL) and intramyocellular lipid (IMCL) did not change in exercised mice. Creatine kinase levels were found
to be significantly higher in exercised MDX mice during the follow-up period and importantly CK levels remained stable, but at high levels, over the whole follow-up period in the exercised MDX mice.
Conclusion: We have described here a longitudinal profile for muscle damage and muscle metabolic changes in MDX mice subjected to chronic exercise and compared this to MDX mice that were not exercised. The extent of the muscle damage by T2-MRI was found to be stable through the follow-up period in the muscles examined while MDX mice that were not chronically exercised showed decreasing signs of muscle damage after the critical period.

‡P60
Muscle MRI quantifies disease activity and severity in hypokalaemic periodic paralysis
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Background: Hypokalaemic periodic paralysis (HypoPP) is a muscle channelopathy characterised by recurrent paralytic attacks and often a progressive fixed myopathy resulting in significant disability. The aim of this study was to quantify both acute and chronic muscle pathology in HypoPP using MRI and correlate these with clinical measures of disease activity and severity.
Methods: Lower limb muscle MRI was performed at 3T in 12 patients with HypoPP (9M/3F, age 42±12y) and 12 healthy controls (9M/3F, age 41±10y). Quantitative sequences included 3-point Dixon fat-water imaging, dual-echo T2 mapping, magnetisation transfer imaging and IDEAL-CPMG fat-water separated T2 relaxometry. Signal intensity from STIR sequences was standardised to a saline phantom. Thigh and calf muscles were analysed on single axial slices respectively 20cm above and 15cm below the knee joint. Clinical data collected included bedside strength assessment, reported attack frequency and the ACTIVLIM functional score.
Results: Quantitative measures (fat fraction, T2, magnetisation transfer ratio and T2 of water component [T2w] were all significantly ($p<0.05$) different in muscles of patients compared with controls at both thigh and calf level. Disease severity measured by bedside assessment of lower limb strength and functional limitation significantly (all $p<0.01$) correlated with all quantitative MRI measures at thigh and calf level (all $p<0.01$) with the exception of ACTIVLIM functional score with T2w. Mean standardised STIR signal intensities were not significantly different and did not consistently correlate with clinical measures. Reported attack frequency did not correlate with any MRI measures.

Conclusion: Lower limb muscles of patients with HypoPP show significant fat infiltration which correlated with measures of disease severity. Increased T2 in the water component of muscle, suggestive of muscle oedema, was also observed, but this was independent of reported attack frequency suggesting MRI provides information concerning disease activity additional to that available from clinical assessment.

P61

Abnormal resting phosphorus metabolism in skeletal muscle in limb girdle muscular dystrophy 2I (LGMD2I)

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Background: Changes in the fat fraction of skeletal muscle have come to be used as a sensitive measure of disease progression but it would be valuable to have non-invasive biomarkers that could be used to assess therapeutic response at earlier stages of disease. Phosphorus spectroscopy is able to measure the concentrations of cell membrane precursors and degradation products at rest, and can measure mitochondrial function under exercise stimulation. This technique may therefore have a useful role in assessing early dystrophic processes.
Aim: To quantify metabolic abnormalities in the skeletal muscle of limb girdle muscular dystrophy 2I patients by phosphorus-31 magnetic resonance spectroscopy.
Methods: 13 LGMD2I patients and 7 controls were recruited through the clinics of the MRC Centre at Newcastle. Phosphorus spectra were acquired on a 3T Philips Achieva with a 14cm radiofrequency coil and a slice selective sequence: spectra were acquired at rest and under exercise. The phosphodiester (PDE), inorganic phosphate (Pi) and phosphocreatine (PCr) resonances were quantified. 3-point Dixon imaging was used to quantify the fat replacement in the gastrocnemius and soleus.
Results: At rest, the patients had a significantly higher concentration of the membrane degradation products, PDE (3.04±0.92mM vs 2.20±0.46mM, p=0.002) and a higher concentration of inorganic phosphate (3.95±0.98mM vs 2.92±0.42mM,p=0.001), while mitochondrial function under exercise was not significantly different (PCr recovery time 36.6±11.4s vs 38.7±9.3s, ns). There was no significant correlation between the abnormalities in PDE or Pi concentration with the degree of fat replacement in the soleus or gastrocnemius muscles.
Conclusion: Phosphorus spectroscopy can detect abnormalities in the concentrations of phosphorus metabolites in skeletal muscle at rest in LGMD2I, in particular the PDE resonances which reflect membrane degradation processes and may be directly related to disease activity. 12 month follow-up measurements will reveal to what degree the abnormalities change across time.

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Magnetic resonance imaging assessments of two doses of drisapersen in the treatment of ambulant boys with Duchenne muscular dystrophy

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Objective: To examine magnetic resonance imaging (MRI) endpoints in a clinical trial of drisapersen, an exon-skipping therapy, in ambulant boys with Duchenne muscular dystrophy (DMD).
Background: DMD is an X-linked recessive disorder caused by mutations in the DMD gene encoding the protein dystrophin. Loss of functional dystrophin results in inflammation, fibrosis, and fatty infiltration of skeletal muscle. Since MRI is sensitive to alterations in muscle chemistry and structure, and can quantify measures related to inflammation and skeletal muscle fat, it has the potential to assess disease involvement and emerging treatment therapies in DMD.

Design/Methods: Subjects received 3 or 6mg/kg/week drisapersen, or placebo, with treatment withdrawn from all groups at week 24. Thirty-four boys attended MRI scanning at baseline and week 24, with four withdrawals at week 48. MRI was used to assess changes in fat infiltration through apparent fat fraction (AFF) measurement, as well as changes in the combined effects of fat infiltration and edematous inflammation through T2-weighted imaging (T2w) and T2 mapping (qT2).
Results: From baseline to both weeks 24 and 48, the 6mg/kg/week group had slower increase in AFF (ranges across the six muscle groups: 0.91-3.78% (n=6) and 0.73-6.56% (n=5)) compared to the untreated group (ranges: 2.66-5.24 % (n=5) and 6.12-8.7% (n=5)).The T2w showed a similar profile as AFF, but qT2 only at week 48.
Conclusion: The small size of the cohort limits the interpretation of the results; however, the natural progression of disease appears to be slowed by treatment with drisapersen in the 6mg/kg/week dose group, as indicated by AFF and T2w. Furthermore, despite withdrawal of treatment at week 24, the apparent treatment effect continued into week 48, suggesting a relatively prolonged response to treatment. With further investigation, these measures may prove to be a suitable imaging marker for assessment of treatments in DMD subjects.

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Utility of muscle MRI in distal myopathies

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Background: Distal myopathies are a diagnostically challenging group of hereditary disorders caused by more than 20 different genes. The pattern of muscle involvement on MRI has been reported for many genes, but the diagnostic utility of muscle MRI in distal myopathies has not been assessed systematically.
Aims: To determine the current diagnostic process in patients with distal myopathy attending muscle specialist clinics at NHNN.
To examine the utility of muscle MRI in this patient cohort.
Methods: Clinical data was collected of patients seen in specialist muscle clinics since 2007 with distal weakness and predominantly myopathic pathogenesis at EMG and muscle biopsy. Patient with confirmed acquired diagnosis were excluded. A review of the published muscle MRI specific pattern in distal myopathies was performed and summarised for each gene. Three examiners, blinded to genetic and clinical data, assessed lower limb muscle MRI scans and scored the image as typical, consistent or different from the literature reported pattern. Seven scans published in the literature were initially assessed, followed by the NHNN patient scans.
Results: 38 patients with distal myopathy were identified, 15 (39%) had a genetically confirmed diagnosis. Muscle biopsy was performed in 89% of patients, with 29% of patients undergoing two biopsied. Muscle MRI was performed more frequently in patients without a genetically confirmed diagnosis (83% vs 40%). Four patients underwent MRI to target the second muscle biopsy; in 2 the second muscle biopsy provided useful additional information. In the assessment of published scans, a median of 2 genes were selected as typical or consistent (range 1-5) with the correct genetic diagnosis included in all cases. Analysis of pattern of muscle involvement in NHNN patients is ongoing.
Conclusion: Muscle MRI in distal myopathy is potentially helpful in guiding genetic testing and selecting site of muscle biopsy.

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Quantitative imaging and spectroscopy to assess clinical outcome in a natural history study of Dysferlinopathy

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Background: Newcastle University, funded by the Jain Foundation, is coordinating an international clinical outcome study for Dysferlinopathy involving 15 worldwide study locations across the USA, Europe, Japan and Australia.
Aims: This study will evaluate genetically confirmed ambulant or non-ambulant Dysferlinopathy patients to determine which outcome measures are most effective at evaluating muscle degeneration in these subjects in a natural history study.
Methods: Medical and physiotherapy assessments are made as well as Magnetic Resonance Imaging (MRI) of the thigh and calf muscles to evaluate muscle degeneration through T2 mapping and fat mapping, and 31P Magnetic Resonance Spectroscopy (MRS) in the tibialus anterior (TA) muscle to determine metabolic changes. The measurements are performed on 6 occasions over 3 years.
Results: 203 patients (104 Female, 99 Male, Age range 11-84) are enrolled. Preliminary analysis in a subgroup of 20 paired subjects (10F, 10M) from Newcastle measured at baseline and at 1 year follow up show a significant ($p<0.01$) increase (>15%) in muscle degeneration of thigh and calf muscles from fat mapping. Significant ($p<0.008$) increases (>42%) of the metabolic markers; inorganic phosphate/phosphocreatine (Pi/PCr) and phosphodiester/total-phosphate (PDE/Ptotal) were also detected.
Conclusion: Muscle fat quantification is sensitive to detect changes in fat infiltration over time. Metabolic changes from baseline can also be detected by 31P MRS. The outcome measures identified during this study will lead to a better understanding of the disease and are essential for the development and success of future clinical trials, which will ultimately lead to new and better therapies.

P65

Accurate slice selection improves responsiveness of quantitative lower limb muscle MRI in CMT1A patients

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Background: Treatment trials for neuromuscular diseases require responsive outcome measures to achieve adequate power. MRI obtained lower limb fat fraction (FF) quantification is a valid, reliable and responsive outcome measure in diseases such as Charcot-Marie-Tooth type 1A (CMT1A). We aimed to determine whether responsiveness (expressed as the standardised response mean [SRM] = mean change/s.d. of change) could be improved, by comparing different analysis approaches.
Methods: We performed lower limb 3T 3-point Dixon MRI, at baseline and 12 months, in fourteen CMT1A patients, and eight healthy volunteers. Muscle FF was obtained across 10 consecutive axial slices (separation 2cm) of the right calf separately in six calf-level muscles. We compared five methods intended to select the optimal slice(s) for analysis, choosing: (a) the numerically central slice; (b) the closest slice to a fixed distance 14cm from the tibial plateau; or calculating the weighted mean of (c) 2, (d) 4 and (e) 6 slices centred 14cm from the tibial plateau. We compared SRMs for each method, for each muscle and also the mean across all calf-level muscles.
Results: In controls, there were no significant FF changes over 12 months. In CMT1A, mean FF increased significantly in all individual muscles by all analyses except method (a). For e.g the extensor hallucis longus (EHL) FF increased by (mean±s.d.): 1.0±5.6, 2.9±4.1, 2.7±3.3, 2.6±2.6, 2.7±2.5 percentage units by method (a), (b), (c), (d) and (e) respectively. SRM was highest in the more sophisticated analyses, being for EHL 0.71, 0.83, 1.00, 1.11 respectively for methods (b), (c), (d) and (e). Mean overall calf-level muscle SRMs were similar to individual muscle values.

Conclusions: We demonstrated that accurate and appropriate selection of slices for analysis can markedly improve MRI outcome measure responsiveness. Increased responsiveness means fewer trial participants required to achieve adequate statistical power to detect treatment efficacy.

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Investigating the effect of dystrophin deficiency on brain function in mouse models of Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive muscle wasting disease caused by mutations in the *DMD* gene. The large cytoskeletal protein dystrophin is also enriched within Purkinje cells (PCs) in the lateral cerebellum. A third of all DMD patients exhibit varying cognitive problems, including severe autism. The dystrophin deficient *mdx* mouse and an *mdx* strain also null for the Cmah gene (*Cmah-/-mdx*), which is similarly mutated in humans, served to analyse the effect of dystrophin deficiency on the brain.
Aim: To investigate brain changes in the *mdx* and *Cmah-/-mdx* models using volumetric *in vivo* MRI measurements in conjunction with histology to investigate structural and cellular abnormalities.
Methods: T1- and T2-weighted magnetization sequences were established to gather brain volumetric measurements at 12 weeks, 25 weeks and 52 weeks of age. Brain weight: brain volume measurements were collected at 25 weeks of age. Control (n=4), *mdx* (n=4) and *Cmah-/-mdx* (n=4) mice (all 39 weeks) were injected intraperitoneally with a bolus of 0.1 mL/10g Evans blue to assess blood-brain barrier (BBB) leakage. PC number was measured following Nissl staining.
Results: Mutant mice showed brain abnormalities by MRI and histology including a reduction of PCs, a feature associated with autistic traits. Enlarged lateral ventricles and increased cerebellar volumes were also evident. An increased brain volume was shown in *Cmah-/-mdx* mice although brain atrophy occurred during aging. MRI changes were subsequently confirmed through Statistical Parametric Mapping. No difference was observed between brain weight and brain volume (25 weeks).
Conclusion: Enlarged lateral ventricles may be caused by impaired regulation of ion transport through the loss of interaction between dystrophin and aquaporin-4, the major CNS water channel. The BBB disruptions suggest defects in fluid handling in the brains of mutant mice. Our investigations have shown that the *mdx* and *Cmah-/-mdx mice* are suitable models to study the effect of dystrophin deficiency on the brain.

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MYO-MRI: The creation of 'Scan Bank'

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Background: In neuromuscular diseases (NMD), the pattern of selective muscle involvement detected by MRI can be pathognomonic and help guide genetic testing, target the optimal muscle for biopsy, and explore disease mechanisms. MRI for diagnostic purposes is not applied in a standardised fashion across neuromuscular centres; often depending on the interest of individual investigators. Patients might miss out on the opportunity to have an MRI potentially contributing to diagnosis or might have an MRI with no added value. Although diagnostic scanning is resulting in an increasing body of MR data, these data may never be published or publicly shared and thus are lost to science.

Aim: To build a searchable web-based database for storage of DICOM files and related metadata as the first step towards an online atlas for muscle imaging. By pooling anonymized images from a broad spectrum of NMD in a secure online database we will enable registered researchers to share MRI studies for research to define the spectrum of selective patterns of pathology and to better understand disease onset, progression and pathophysiology. The curated images that are representative of different phenotypes will then be used in the formation of a publically accessible imaging atlas for NMD.
Methods and Results: The authors, in conjunction with Zitelab, have developed the '*ScanBank',* an online database with a web based user interface to enable upload and viewing of DICOM studies on an ongoing basis. It will contain a built-in open source DICOM viewer and will anonymise studies on upload for security. In a second phase of development the database will be extended to enable data to be entered using clinical ontologies (e.g. Orphanet Rare Disease Ontology and Human Phenotype Ontology) and will be connected to omics research data via RD-Connect.
Conclusion: By developing a searchable web-based database for storage of DICOM files we are facilitating data sharing for research into selective muscle involvement in NMDs and enabling development of an online atlas of neuromuscular imaging that will enhance the knowledge of scientists and healthcare professionals and ultimately benefit patients with neuromuscular disorders.
This project is part of MYO-MRI COST-Action BM1304: "Applications of MR imaging and spectroscopy techniques in neuromuscular disease: collaboration on outcome measures and pattern recognition for diagnostics and therapy development".

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Diffusion MR study at 7T of hind limb muscles in dystrophic mice

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Background: Muscle fiber size has higher variability in dystrophic (*mdx*) than *wild-type* (*wt*) mice, as typically assessed by histology.
Diffusion MRI is a non-invasive technique sensitive to tissue structure at the microscopic level which may be clinically useful for assessing dystrophic muscle.
Aims: To investigate the feasibility of diffusion MRI for assessing differences in the microstructure between hindlimb muscles of dystrophic and *wt* mice.
Methods: Male *mdx* (n=17) and *wt* (n=17) mice of three groups of age were used for *in vivo* and *in vitro* MRI investigation of hindlimb muscle microstructure. The MRI protocol included multi-slice-multi-echo images for T_2 determination, and diffusion-weighted imaging. Six diffusion times (Δ), ranging from 25 to 350 ms, were explored with diffusion gradient applied along and across muscle fiber direction. Apparent diffusion coefficients (*ADC*) were calculated in hindlimb muscles of each mouse. Following MRI, mice were sacrificed and excised limbs fixed in formalin. *In vitro* measurements were performed using the same MRI protocol. Fixed limbs were decalcified, sections stained with H&E and muscle fiber diameter measured by ImageJ.
Results: Compared to *wt* mice, *ADC values* of hindlimb muscle in *mdx* mice were significantly different at long Δ. A significant decrease in water diffusivity was observed with increasing mouse age. A correlation between T_2 and *ADC values* of hind limb muscles in *mdx* and *wt* mice was also shown. *In vitro* data showed the same diffusivity pattern of *in vivo* data but with lower ADC values. A broader distribution of muscle fiber size in *mdx,* compared to *wt* mice, was confirmed by histology.
Conclusion: This study demonstrates the ability of diffusion MRI to discriminate dystrophic and healthy muscle when investigated using long diffusion times.

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Manganese enhanced muscle MRI as a sensitive outcome measure of dystrophin restoration in the mdx mouse

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Background: A central component of dystrophic pathology in mdx mice as well as DMD patients is a persistent increase in steady-state calcium levels in muscle. Manganese acts as a contrast agent in T1 weighted MRI and is thought to be taken up by cardiac and skeletal muscle by the same receptors as calcium. We have previously shown that manganese-enhanced MRI (MEMRI) is a sensitive marker for cardiac pathology in mdx mice. Phosphorothiodate Morpholino (PMOs) have been shown to induce exon skipping and thereby dystrophin expression in the mdx mouse.
Aim: To determine whether MEMRI is a sensitive outcome measure of dystrophin restoration in PMO treated mice.
Methods: Mice were given three weekly injections of one of two doses of PMO. Two weeks following their last injection, they underwent MEMRI. Mice were sacrificed and tissues removed for immunofluorescence. Dystrophin and laminin double staining was carried out.
Results: Mice showed a PMO dose dependant reduction in MEMRI contrast with an increase in dystrophin expression.
Conclusions: These data suggest that muscle MEMRI may be a valuable tool for longitudinal studies on dystrophin restoration in mdx mice.

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MRI quantitation of muscle water with IDEAL-CPMG

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Background: Quantitative MRI is becoming increasingly important for new neuromuscular disease therapy trials. Measuring muscle water changes independently of fat concentration is vital because water changes often occur early in disease and are potentially reversible. The measured MRI muscle T2-relaxation time is conventionally presumed to be that of water (T2w), although values are often heavily influenced by fat. The novel IDEAL-CPMG method combines chemical-shift fat-water separation with T2-relaxometry to allow T2w measurement independent of fat content.
Aims: We evaluated IDEAL-CPMG reproducibility by repeated measurements in healthy individuals, and assessed the relationship between muscle T2w and fat-fraction (f.f.) in patients demonstrating both water and fatty changes.
Methods: The calf muscles of 11 healthy adults (9 male, 40.6±10.3yrs) were scanned twice, 4-weeks apart, with 3T IDEAL-CPMG-MRI. Fat and water signals were separated using a 7-peak fat lipid spectrum model and the f.f. calculated. T2w was determined by least-squares fitting a mono-exponential decay function to 15 spin-echoes. Manual segmentation was used to determine mean muscle values. 11 hypokalemic periodic paralysis (HypoPP) patients (7 male, 41.4±12.0yrs) were scanned once and data analysed in the same manner.
Results: The 4-week-repeat Bland-Altman mean bias and limits-of-agreement were T2w:(0.16, -2.58, 2.90)ms and f.f:(0.04,-2.79, 2.87)% with all paired t-tests non-significant, indicating good reproducibility. Mean T2w and f.f. were 30.4±1.3ms and 4.3±1.2% in control tibialis anterior muscles. There was no Spearman correlation between T2w and f.f. in controls ($p=0.9$) and a weak association ($\rho=0.54$; $p<0.01$) in HypoPP patients.

Conclusions: IDEAL-CPMG provides sensitive, reproducible measures of muscle water T2 without substantial contributions from fat. Residual correlation between f.f. and T2w may reflect concurrent pathological changes, i.e. true T2w increases accompanying f.f. increases in HypoPP. IDEAL-CPMG may be advantageous over alternative approaches for quantifying muscle water changes, providing early indices of disease progression, essential for neuromuscular disease therapeutic trials.

Muscle Channelopathies and Myasthenia Gravis

‡P71

Correlation of the functional properties of ClC-1 variants with inheritance pattern of clinical symptoms

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Background: Myotonia congenita (MC) is caused by loss-of-function mutations of ClC-1 chloride channels that result in increased excitability and failure of muscle to terminate contraction following activity. Mutations occur throughout the channel sequence and may be inherited in a dominant, semi-dominant or recessive manner. This makes interpretation of new sequence variants and genetic counselling of patients difficult.
Aims: 1. To functionally characterise missense mutations identified in patients referred for genetic testing of *CLCN1* 2. To develop a framework for assessment of likely pathogenicity and risk of dominant inheritance.
Methods: Clinical data was prospectively collected. As segregation data was not sufficient to be certain of variant inheritance and penetrance in all cases, the associated inheritance pattern of myotonic symptoms was determined according to available clinical and genetic information. Identified *CLCN1* variants were expressed in *Xenopus* oocytes in homomeric and simulated heterozygous conditions. The crystal structures of CmClC and ClC-5 were used to generate a homology model of ClC-1.
Results: 81 of 83 missense variants could be categorised into four different functional groups: wild-type like, functionally recessive, voltage-shift 1 and voltage shift 2. The functional groups correlate well with the clinical profiles of patients. Those with wild-type like variants were significantly more likely to have an uncertain relation of the variant to myotonic symptoms (p= 0.0003). Mapping the variants onto the molecular model of ClC-1 reveals a disproportionate clustering of residues associated with a dominant inheritance of myotonic symptoms in the region from helix B to the end of the IJ linker (p= 0.0002).
Conclusion: Combined clinical, functional and genetic data with molecular modelling has enabled us to develop a framework for analysis of ClC-1 variants. This framework has yet to be verified outside of our dataset but forms an important first step in providing more detailed guidance for the interpretation of novel ClC-1 variants.

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***SCN4A* mutations in a patient with congenital myopathy**

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Background: Gain-of-function mutations in the $Na_V1.4$-encoding gene *SCN4A* are responsible for the allelic disorders paramyotonia congenita and hyper-/hypokalemic periodic paralysis. Here, we report a novel phenotype of congenital myopathy associated with two specific *SCN4A* mutations.
Aims: To characterize the electrophysiological and pathological effects of *SCN4A* mutations in a patient with congenital myopathy.
Patient Details: The proband is now a 14-year-old girl. She presented at birth with floppiness and poor feeding. Later she displayed delayed motor development milestones and recurrent chest infections. Nocturnal non-invasive ventilation was required from age six and spinal surgery for progressive scoliosis at age 13. Examination at this time revealed subgravity axial and hip girdle muscle power. Serum CK and single fiber EMG were normal. Muscle biopsy revealed small slow myosin fibers consistent with congenital myopathy. Muscle MRI demonstrated bilateral symmetric involvement of sartorius, adductor magnus and soleus without edema.
Results: The previously reported R1135C and a novel R104H *SCN4A* mutation were identified by whole-exome sequencing, and confirmed with Sanger sequencing. Asymptomatic parent DNA samples are at present unavailable for sequencing. HEK293 cells transiently transfected with R104H cDNA expressed no sodium currents – demonstrating a complete loss-of-function. The homozygous R1135C mutation has recently been reported in a patient with hypokalemic periodic paralysis. R1135C channels conduct gating pore currents. R104H leads to loss-of-function. In order to determine how these effects underlie the permanent weakness in the patient, it will be important to determine if the mutations are located on the same allele.
Conclusion: This is first report of a total loss-of-function in $Na_V1.4$, and the first identified case of congenital myopathy caused by *SCN4A* mutations. This finding may account for similar phenotypes in patients without diagnosis, and indicates that *SCN4A* mutations may be associated with a much wider spectrum of clinical and pathophysiological features than previously anticipated.

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A novel drug model of hypokalaemic periodic paralysis in neonatal cell cultures

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Background: Hypokalaemic periodic paralysis (HypoPP) is an autosomal dominant disease resulting from mutations in the alpha subunit of skeletal muscle voltage gated sodium or calcium channels. The direct effects of these mutations are well characterised, leading to an understanding of the initial disease phenotype (attacks of paralysis). However, many patients develop a progressive myopathy and a reduction in attack frequency. We propose that dysregulation of calcium signalling and downstream effects on mitochondrial function play a central role.
Barium and gramicidin have both been used in mouse tissue to simulate the 'paradoxical depolarisation' observed in HypoPP. However these drugs have not been tested in combination or in cultured cells.
Aims: To develop a drug model that may be used to investigate indirect effects of the HypoPP mutation such as changes in calcium handling and mitochondrial function. In this model barium and gramicidin are used in combination in cultured neonatal rat cells. We aim to also use these in isolated single fibres from control mice and in control immortalised human myoblast cultures.
Method: Initially the effects of barium and gramicidin on membrane potential are being tested electrophysiologically in order to determine the most suitable drug concentrations for this investigation. This is done with each drug in isolation, and with both in combination.
Once suitable drug concentrations are determined, drugs will be administered during cell culture. Potassium will be lowered in both treated and control cells. It will be lowered enough to produce 'paradoxical depolarisation' in the treated cells but not in control.

Cells will be returned to normal culture conditions before taking calcium handling, oxygen consumption and mitochondrial membrane potential measurements. This should indicate whether or not the treatment has downstream effects after wash-off, and consequently if it is a suitable model for investigating the indirect effects of HypoPP mutations.
Results: In progress.

P74
A new sodium channel myotonia (SCM) mutation in the Nav1.4 DII-S4S5 linker
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Background: Mutations in the voltage-gated sodium channel Nav1.4 may produce different gain of function effects, each of which can predispose to a distinct clinical phenotype. Pathogenic mutations in the DII-S4S5 linker of Nav1.4 are usually associated with episodic weakness through a combination of enhanced activation and disrupted slow inactivation. Slow inactivation seems to play a major role in determining the episodic weakness phenomenon.
Aims: To describe clinical and functional effects of a new *SCN4A* mutation located in the DIIS4S5 linker with a pure myotonic phenotype.
Methods: A 39 year-old-man, presented with a 29 year history of muscle stiffness exacerbated by cold and fasting. No episodes of weakness have been reported. Neurological examination showed lid-lag with mild paramyotonia and upper limb percussion myotonia. A variant c. G2095A (p.A699T) was found on *SCN4A* gene. The biophysical properties of the mutant Nav1.4 channels were evaluated by whole-cell voltage-clamp analysis of HEK293 cells transiently transfected with WT or A699T Nav1.4 channel.
Results: The mutation did not affect the voltage dependence of activation (WT=-18.7±0.6mV, n=8; A699T=-17.1±1.3mV, n=9) but caused a small depolarized shift of the V_{mid} of the voltage dependence of inactivation (WT= -64.3±1.0 mV, n=7; A699T= -61.8±0.5 mV, n=10; $p<0.05$). Moreover there was a significant slowing down in the time constant of fast inactivation between WT and A699T ($p<0.05$) (WT n=7, A699T n=10) at when measured at -10 mV. Steady state of the voltage dependence of slow inactivation was enhanced in mutant A699T (WT=-43.8±2.1mV,n=5; A699T=-56.9±1.8mV,n=3; $p<0.005$).
Conclusion: A699T mutation likely causes a myotonic phenotype through an alteration of fast inactivation. The enhancement of slow inactivation in A699T mutation may explain the absence of weakness in our patient. Other mutations in the same domain often disrupt slow inactivation and predispose the patients to episodic weakness.

P75
Rituximab treatment in two subacute resistant bulbar myasthenia cases
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Background: Myasthenia Gravis is a multiform disease because of a wide range of symptoms whose expression can vary in time, presentation, and entity. Bulbar signs such as breathing impairment and dysphagia can mimic other acute syndromes and lead to misdiagnosis. They and are also often associated with more aggressive myasthenia, and less responsive to conventional therapy.
Aims: Aggressive myasthenic syndromes need a fast acting drug when conventional first line therapy fails.
Patients: A 72 old woman and a 74 old man attended our Emergency Unit complaining of diplopia and dysphagia; rynolalia was evident during speaking and briefly they also manifested breathing impairment.

Results: A Pyridostigmine test was only slightly positive and both patients had little improvement with pyridostigmine therapy. Acetylcholine receptor antibodies were positive (very high titre in the man's case) and a repetitive nerve stimulation test was consistent with a neuromuscular junction disorder. Prednisone 1mg/Kg/day, plasmapheresis and Immunoglobulin could improve symptoms for a short time, a target we were only able to maintain using rituximab 600 mg every week for the first 4 infusions, then every month.
Conclusions: These clinical cases evidence how rituximab could be a faster acting drug alternative to other immunosuppressant drugs and should always be considered in difficult to treat and poorly responsive myasthenic patients

P76

Exome sequencing in congenital myasthenic syndromes

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Background: Congenital myasthenic syndromes (CMS) are caused by inherited defects at the neuromuscular junction which can be presynaptic, synaptic or postsynaptic. The use of exome sequencing has expedited the identification of novel causal mutations in CMS through use of platforms such as clinical sequence analyser (CSA) provided by deCODE Genetics (Iceland). Using this platform, we have analysed the exomes of monozygotic twins and an affected sibling with clinically confirmed CMS.
Aims: To identify potential CMS causing mutations within a trio with a CMS phenotype. To investigate the function of potential causative variants in a Zebrafish model.
Methods: Whole exome sequencing, alignment to the reference sequence and variant calling was carried by deCODE, Iceland. Variants were then analysed using their proprietary software CSA. We excluded variants based on allele frequency (<0.01), effect on the protein (VEP: lowest to low) and the relevant inheritance model (autosomal recessive).
Results: After the first exclusion phase 126 variants were identified. Exclusion of variants in cis and with a low coverage allowed a reduction in the number of potentially damaging variants. The gene function of the variants also excluded a further 34 variants. After reviewing current literature relating to the remaining 6 variants, a candidate gene was selected based upon its possible role in the neuromuscular junction. This role is to be investigated using functional analysis in cell culture and also knock down morpholinos in zebrafish.

P77

An investigation into the side effect profile, safety profile and perceived efficacy of mexiletine for the treatment of myotonia in a cohort of patients with dystrophic and non-dystrophic myotonia

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Background: Mexiletine is an evidence-based treatment for myotonia. Its potential for deleterious cardiac effects in the non-cardiac population may be overestimated.
Aims: Determine clinical and electrocardiographic adverse effects and efficacy of mexiletine in our myotonic cohort.
Methods: Retrospective review of 71 patient records, significance assessed using paired t-test or one way ANOVA with *post hoc* unpaired t-testing.
Results: There were no serious adverse patient events. The most common side effect was dyspepsia that caused 3 patients to stop mexiletine despite dyspeptic treatment. Paired assessment showed no significant change in QTc on or off Mexiletine at any dose. 16 patients were referred to a Cardiologist because of concern over

Mexiletine therapy: all were felt safe to continue Mexiletine. Two patients with documented inefficacy of Mexiletine previously and 5 who had stopped due to side effects found Mexiletine effective and tolerable on retrial. Patients with myotonia secondary to *CLCN1* missense mutations required significantly higher doses of mexiletine than those with *SCN4A* missense mutations.
Conclusions: Mexiletine is a safe and effective treatment in patients with myotonia. Dyspepsia is a common and troublesome side effect. A retrial of Mexiletine therapy is worth considering in apparently refractory patients. *CLCN1* patients may require higher doses for efficacy.

P78

Developing validated specific home based assessment tools for monitoring fluctuations in fatigability and muscle performance in adult and paediatric myasthenia patients

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People with myasthenic conditions have symptoms of muscle weakness and fatigue, which characteristically fluctuate and often vary week to week, day to day and typically within the same day. Ideally, clinic assessments should be supplemented by a tool which can detect changes in functional ability at home. This may help monitor the effects of medication and provide early warning of illness or impending crisis enabling patients to seek medical advice. Without timely medical review, myasthenic crisis could develop leading to hospitalisation.
The aim of this research is to develop and validate specific home based assessment tools for monitoring fluctuations in fatigability and muscle performance in adult and paediatric myasthenia patients.
The first step is to gain further insight and identify physical challenges patients have to overcome when living with myasthenia. This will be achieved by conducting semi structured interviews with the patient population. 10 adults and 5-10 children, between the ages of 5 and 18 years, are being recruited for interview.
Following the interviews, recurrent themes will be extracted and participants will be asked to rate how much they agree with each theme using the Delphi Method of qualitative research.
Based on the findings from the interviews, assessment methods/tools will be selected that correlate to the themes identified. These will be selected from validated, novel and exploratory functional assessments to determine the most suited to this population.
Participants will complete the functional assessment when attending hospital clinic appointments. These assessments will be further analysed for their use in the myasthenia population using modern psychometric techniques via item response theory with the Rasch Measurement Method. A selection of the assessments will then be carried forward and translated into a home assessment tool.
Interview data is currently being collected and preliminary results will be presented at conference.

P79

Integrins are required for synaptic transmission and development of the neuromuscular junction

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Background:
Development of the neuromuscular junction (NMJ) depends on interactions between proteins in the nerve terminal, muscle fibre and in the synaptic cleft. Laminins play a central role, as they bind to presynaptic voltage-

gated calcium channels and to dystroglycan in the muscle fibre. Integrin-α3 is a laminin receptor expressed in the presynaptic active zones, the sites of neurotransmitter release across the synaptic cleft.
Aims and methods: To determine the function of integrin-α3 at the NMJ, using mice with a genetic ablation of the integrin-α3 gene.
Results: At embryonic day E18.5, $\alpha3^{-/-}$ mice present with abnormal assembly of active zone proteins and reduced synaptic transmission, as determined by electrophysiology. Ultrastructural studies reveal defective deposition of the basal lamina at the synaptic cleft. As $\alpha3^{-/-}$ mice die at birth due to the failure of multiple systems, we used $\alpha3^{+/-}$ mice to study the role of this protein in postnatal maturation of the NMJ. In adult $\alpha3^{+/-}$ mice, we find aberrant NMJ morphology and reduced expression of several active zone proteins.
Conclusion: Integrin-α3 is important for the assembly of active zones and of the synaptic basal lamina. The data suggests that defects in this protein may be associated with defect of neuromuscular junction transmission in humans.

Peripheral Nerve Disease

‡P80

Hereditary sensory neuropathy type 1 (HSN1) secondary to SPTLC1/2 mutations: investigating the role of deoxysphingolipids in the pathogenesis

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Background: HSN1 is a progressive sensory motor neuropathy leading to profound loss of sensation with variable motor involvement. Mutations in the genes *SPTLC1/ 2*, which encode for subunits of the enzyme serine palmitoyltransferase, alter its substrate specificity leading to the build up of deoxysphingolipids (dSLs). These are postulated to be neurotoxic.
Aims: To determine if dSLs are neurotoxic in a mammalian in-vitro model and whether the toxicity reflects the clinical phenotype.
Method: Cell survival was assessed in primary motor neuron and dorsal root ganglia cultures following treatment with dSLs (1-deoxysphinganine and 1-deoxymethylsphinganine) for 12, 24, 36 and 48 hours. Immunocytochemistry with Beta III Tubulin was used to label the neurons. Differential toxicity within sensory neuron sub-populations was assessed after 12 hours of treatment using CGRP (small, peptidergic neurons) and neurofilament 200 (large, myelinated neurons).

Results: Both 1-deoxysphinganine (DSP) and 1-deoxymethylsphinganine (DMSP) appear to be neurotoxic to motor neurons and DRGs. DSP is more neurotoxic. Sensory neurons are more vulnerable to the toxicity with significant reduction in cell count seen earlier and greater reduction seen when compared to motor neurons. Reduction in DRG neurite outgrowth is seen in the early stages of dSL induced toxicity. The CGRP positive neurons are more susceptible to the toxic effects when compared to the neurofilament positive neurons.
Conclusions: Deoxysphingolipids are neurotoxic to mammalian motor neurons and sensory neurons with the latter being more vulnerable. There is differential toxicity within the sensory sub-populations with the peptidergic, CGRP positive neurons being more susceptible than large, neurofilament positive neurons. Overall, these findings illustrate that the pattern of deoxysphingolipid induced neurotoxicity in this in-vitro model mirrors the clinical phenotype seen in HSN1 patients. Future work is now focused on investigating the targets of this toxicity and whether these dSLs or their downstream products are responsible for the toxicity.

‡P81

Nidogens define a novel pathway for ligand entry and signalling at the neuromuscular junction

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Impaired axonal transport in specific neuronal populations has been implicated in a number of neuromuscular diseases such as SMA, ALS and CMT. The nontoxic carboxyl-terminal fragment of the tetanus neurotoxin, HcT, is widely used as a marker of retrograde axonal transport in neurons. *In vivo*, HcT is endocytosed at the presynaptic terminals of motor neurons and undergoes retrograde transport and trancytosis into the CNS. Recent work has indicated that the internalisation and transport of HcT is dependent on the presence of nidogens, known also as entactins, key components of basement membranes. Nidogens bind HcT and may be cotransported in endosomal compartments that have been shown to contain neurotrophin receptors. As certain isoforms of nidogen appear to be specific to the neuromuscular junction this protein may represent a novel mechanism to enable selectivity not only of HcT binding, but also of neurotrophin signalling. The main aim of this project was to develop methods investigate the physiological role of nidogens in motor neurons *in vitro* and *in vivo*. A retrograde transport assay previously established in the lab was further developed allow confocal imaging of nidogen, HcT and neurotrophin receptor cotransport. Mammalian primary motor neurons were cultured in microfluidic chambers to achieve fluidic isolation of the axon terminals and to separate individual axons. Furthermore, I fused a modified biotinylation enzyme, BirA, to HcT to investigate the signalling complex associated with nidogens. This technique called proximity-dependent biotinylation will allow the specific biotinylation of novel components of the nidogen-HcT complex, enabling their isolation and identification by mass spectrometry. The identity of these proteins will reveal the physiological function of nidogen transport in motor neurons. Further investigation into the novel signalling pathway used by nidogen may therefore reveal promising targets for the restoration of axonal transport and as biomarkers of disease.

P82

Clinical and genetic spectrum of X-linked Charcot-Marie-Tooth disease due to mutations in the 5' untranslated region of *GJB1*.

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Background: Mutations in the 5' untranslated region (5'-UTR) of *GJB1*, including the promoter P2 region and exon 1B, have been reported to cause X-linked Charcot-Marie-Tooth disease (CMTX1; MIM 302800). These mutations have been estimated to account for only 0.01% of all CMTX1 cases, and their clinical spectrum still remains incompletely evaluated.
Aim: To assess the clinical and genetic characteristics of patients with CMTX1 due to mutations in *GJB1* 5'-UTR.
Methods: We analysed detailed demographic, clinical, pedigree and neurophysiological data of patients with CMTX1 and mutations in *GJB1* 5'-UTR. Mutations were identified by bidirectional sequence analysis of *GJB1* from bases -550 to +1.
Results: A total of 16 individuals from 6 different families were included in the study. Five different pathogenic or potentially pathogenic mutations in the GJB1 5'-UTR were detected. The previously reported mutation c.-17G>A was detected in 3 families. Other two previously reported mutations, c.-103C>T and c.-146-25G>C, were detected in two separate families. A novel mutation predicted to disrupt an EGR2 binging site was found in one individual.
Conclusions: Analysis of the data is still underway and results will be presented at the conference.

P83

Aminoacyl-tRNA synthetases (ARS) related inherited axonal neuropathies in the North England cohort of CMT patients

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Background: Axonal form of Charcot Marie Tooth disease (CMT2) is a clinicogenetically heterogeneous group of hereditary sensorimotor neuropathies. There is a phenotypic overlap between CMT2 and distal hereditary motor neuropathy (dHMN), a subgroup of rare inherited motor axonal neuropathies with no significant sensory involvement. Mutations were described in genes encoding axonal proteins. Aminoacyl-tRNA synthetases (ARS) are essential enzymes for translation the genetic code into proteins. There have been six ARS implicated in CMT2 and dHMN related axonal pathology. Majority of the mutations were described in glycyl-tRNA synthetase (GARS).
Aim: To analyse the pheno- and genotypic characteristics of patients with GARS and alanyl-tRNA synthetase (AARS) mutations indentified in a cohort of CMT2/dHMN from the North East of England.
Patients: In a large cohort of 438 CMT patients, multi-gene panel assay and whole exome sequencing confirmed 4 clinically suspected GARS neuropathies and identified AARS mutation in 4 CMT2 patients.
Results: All patients carrying GARS mutations presented with an early adulthood onset upper limb predominant axonal motor neuropathy associated with mild motor weakness in the lower limbs and minor sensory involvement. A characteristic wasting in the first dorsal interosseus hand muscles led to a split hand malformation. Identification of heterozygous novel mutations in the GARS gene supported the clinical diagnosis. The previously described c.986G>A recurrent heterozygous AARS mutation was present in all 4 CMT2 patients. All had a moderate predominantly motor axonal neuropathy with variable age onset. However hand involvement was present, the lower limbs were more affected with minor sensory changes.
Conclusions: Mutations in ARSs are implicated in dominant axonal CMT. Despite the large number of variants and multiple theories of underlying pathology, GARS related clinical phenotype has remained quite distinctive. The recurrent AARS variant is associated with a more heterogeneous phenotype due to a methylation mediated loss of function mechanism.

P84

CIDP in overlap syndrome autoimmune hepatitis

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Background: CIDP is a disimmune peripheral nerve disease. Immunological etiology is based on various experimental data and pathological reports, often associated to the presence of myelin associated glycoprotein antibodies.
Aims: There's often an association among disimmune diseases in the same subject and their time course can be variable.
Patient: A 50 years old woman, diagnosed with overlap syndrome autoimmune hepatitis complained of paresthesia and weakness of the arms and legs, 4 years after the onset of liver disease, which was diagnosed as AIDP, with a subsequent relapsing remitting course CIDP like.
Results: Spinal fluid examination resulted in protein-cells dissociation while electro diagnostic studies evidenced demyelinating polyneuropathy. The clinical course revealed a CIDP type neuropathy and steroids were prescribed along with azathioprine as a steroid sparing drug. After cholecystectomy surgery due to autoimmune cholangitis , the patient became HCV positive showing a great increase in transaminases, this therefore lead to a reduction of steroids along with a tapering off of azathioprine, leading to a cessation of both drugs. This change in drug application lead to a worsening of the patient, which could only be rectified by the reimplementation of the steroid.

Conclusions: Steroid therapy rapid response in a CIDP associated to autoimmune disease proves once again the disimmune mechanism in CIDP and it proves probable autoimmune common ethiology of disimmune pathologies coexisting in the same subject.

P85

Survey of pregnancy in patients with Charcot-Marie-Tooth disease and related hereditary neuropathies

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Background: Charcot Marie Tooth (CMT) disease is the most common inherited peripheral neuropathy. Patients frequently ask about whether pregnancy will affect their CMT, whether CMT will affect their pregnancy, what type of delivery they should have and whether they or their child will have a higher risk for any of the complications of pregnancy or delivery. However very few studies have been published on how CMT can affect pregnancy, birth and the newborn and information is largely restricted to small case series and single case reports. Currently guidelines for the management of pregnancy, delivery and postnatal care in CMT patients are not available.

Aim: to assess the impact of pregnancy on CMT and to assess how CMT affects pregnancy, the delivery and the care of the new born baby.

Methods: We designed a questionnaire with expert help from an obstetrician with a special interest in pregnancy in patients with medical conditions. The questionnaire includes questions on impairment, falls, pain, fatigue and respiratory complications prior, during and after pregnancy; type of delivery, possible complications, anaesthesia and difficulties looking after the baby in the first couple of months.

Results and conclusions: The survey is currently ongoing. The data acquired from the questionnaire will provide valuable information on current practice and will inform future guidelines and standard of care in Charcot Marie Tooth disease.

P86

Management of the orthopaedic complications in Charcot-Marie-Tooth disease patients

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Background: Charcot-Marie-Tooth (CMT) disease is the most common inherited peripheral neuropathy. Foot deformities are frequent complications in CMT patients and orthopaedic surgery is often required. However there are no evidence based guidelines on the type or timing of the surgery. Only few studies have described the long-term results of surgical procedures and evidence regarding optimal surgical management of these patients is lacking.

Aims: To prospectively study surgical management of CMT patients attending our centre.

Methods: Collection of data and assessment of patients before and for 2 years after surgery.
Data included: history of ankle instability, pain, skin condition, details of physiotherapy and orthotic management, assessment of lower limb strength, Charcot-Marie-Tooth Examination Score (CMTES), Foot Posture Index, ankle dorsiflexion range of movement, quality of life questionnaire (SF-36) and specific questionnaires (foot index and Manchester-Oxford foot questionnaire, modified fatigue severity scale and modified falls efficacy scale), details of surgical procedures.

Results: 11 CMT patients were evaluated prior to surgery, 8 patients were assessed after 1 year and 2 patients after 2 years from surgery. All patients had significant improvement of foot alignment and pain after 1 year. Analysis of prospective assessments is still ongoing.

Conclusions: The results of the study will help develop orthopaedic intervention guidelines and inform questions for further research.

P87

Survey of current management of orthopaedic complications in Charcot-Marie-Tooth disease patients

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Background: Foot deformities, namely forefoot cavus, clawtoes and hindfoot varus, are frequent complications in Charcot-Marie-Tooth disease (CMT) patients. Orthopaedic surgery is frequently undertaken in CMT patients to correct severe foot deformities. Treatment is determined by the age of the patient and the severity of the deformity and is usually indicated to prevent further deterioration. However there are no systematic studies on the surgical management and the current approach varies between centres in the same country and between different countries.
Aim: To test the hypothesis that current surgical approach to management of orthopaedic complications in CMT is variable even at CMT specialised centres.
Methods: A survey including two different case scenarios of typical CMT patients (one adult and one child) was designed and addressed to orthopaedic surgeons performing surgical procedures for foot deformities in CMT patients attending centres participating in the Inherited Neuropathy Consortium (INC).
Results: 16 surgeons working in different specialised centres (UK, Italy, Australia and US) answered the survey. The majority of the surgeons were ankle and foot surgeons (94%) and 25% of the surgeons were adult only whereas 50% were paediatric only. Only 19% of the surgeons were both paediatric and adult surgeons. Interestingly there was a marked variation in practice among surgeons with only 2 paediatric surgeons and no adult surgeons agreeing a surgical approach for specified clinical scenarios.
Conclusions: These findings confirm the hypothesis that there is currently a variable approach to the orthopaedic management of CMT patients and the results will inform future studies on orthopaedic interventions in CMT patients.

P88

Charcot-Marie-Tooth and Centronuclear myopathy induced mechanistic impairment in endocytosis

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Background: Dynamin 2 (DNM2) is involved in a wide range of cellular processes such as the scission of endocytic vesicles, membrane and cytoskeletal dynamics. Mutations in the *DNM2* gene have been associated with Charcot-Marie-Tooth (CMT) and Centronuclear Myopathy (CNM) in a tissue specific manner. Although mutations in DNM2 have been characterised biochemically, little is known about their influence on the dynamics of nanoscale morphological changes taking place in individual trafficking events. Such knowledge is critical for understanding of disease-related perturbations.
Aims: To determine the role of DNM2 mutations in endocytic vesicle formation and internalisation.
Methods: Correlative Scanning Ion Conductance Microscopy (SICM) and fluorescence confocal (FC) microscopy developed in our lab allows live imaging at super resolution. We applied this technique in tandem with biochemical assays to characterise the temporal and spatial recruitment of mutant dynamins and their effect on formation and internalisation of individual endocytic pits.
Results: Topographical and confocal data demonstrated R465W DNM2 co-localise with nascent pits in a stable manner, which gradually flattened and dissipated without scission. We report the aberrant pit formation and clustering of R552H DNM2. Both mutations are observed to adversely affect the average lifetime of clathrin coated pits compared to wild type DNM2.

Conclusion: The applications of correlative SICM and FC live imaging have allowed for the first time the characterisation of DNM2 mutants in living cells at single vesicle level. The data acquired provides direct evidence that DNM2 mutations alter the kinetics of pit formation and internalisation in a mutation specific manner.

P89

Charcot Marie Tooth Disease Natural History Study: evaluation of the CMT paediatric scale in the UK cohort

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Background: The Charcot Marie Tooth (CMT) Natural History Study is a multi-centre international study involving centres in the UK, USA, Italy and Australia belonging to the Inherited Neuropathy Consortium (INC). As part of the project a paediatric scale, the Charcot Marie Tooth Disease Paediatric Scale (CMTPeds) was designed and validated to measure disability and progression of disease in children with CMT aged 3 to 22. The scale is a composite scale including 11 items evaluating strength, dexterity, sensation, gait, balance, power, endurance.
Aim: To characterise the natural history of the UK cohort of CMT children and to assess significant changes in individual items included in the CMTPeds over time.
Methods: Annual assessments using the CMTPedS scale has been carried out at yearly assessments on patients from 3 to 22 years of age. The CMTPeds include: Lunge test, Foot Posture Index, Functional dexterity test, myometry to assess hand grip, foot dorsiflexion and plantarflexion strength, pin prick and vibration testing, balance testing using the BOT-2 balance component, six minute walk test and long jump.
Results and Conclusions: Currently 71 children have been recruited in the UK centres (Dubowitz Neuromuscular Centre and National Hospital for Neurology and Neurosurgery). 71 children with age comprised between 6 and 22 were assessed at baseline, 37 children had 1-year follow up, 19 children had 2-year follow up, 6 children had 3-year follow up and only 1 had 4-year follow up. 58 (82%) children had a genetically confirmed diagnosis of CMT (74% CMT1A, 0.4 % CMT1B, 10% CMTX, 0.5% CMT2A). Analysis of changes over time of individual items of CMTpeds is still in process and final results will be presented at the conference.

P90

Riboflavin transporter neuronopathy

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Background: First described in 1894, Brown-Vialetto-Van Laere (BVVL) syndrome is a rare, autosomal recessive neurodegenerative disorder characterised by bilateral sensorineural hearing loss, cranial nerve palsies, respiratory insufficiency and severe sensorimotor neuropathy. Most infants with this condition rapidly become ventilator-dependent and die during childhood. Mutations in two riboflavin transporter genes, SLC52A2 and SLC52A3, have recently been shown to underlie a number of severe cases of BVVL syndrome.
Aims: Our aims were to investigate: A) the scope of mutations occurring in these genes; B) the *in vitro* effects of SLC52A2 mutations on cellular energy metabolism and mitochondrial function in fibroblasts of patients; and C) the *in vivo* consequences of the loss of the SLC52A3 homologue in the fruit fly, *Drosophila melanogaster*.
Methods: We used Sanger sequencing to screen 116 patients exhibiting cranial neuropathies and sensorimotor neuropathy +/- respiratory insufficiency. We then performed functional assays and measured activities of mitochondria respiratory complexes in patients with SLC52A2 mutations. We also employed an RNAi-mediated gene knockdown of the *Drosophila* SLC52A3 homologue to recapitulate the loss-of-function phenotype of BVVL.

Results: We identified 18 BVVL cases summing 5 SLC52A2 and 14 SLC52A3 pathogenic mutations, of which 4 SLC52A2 and 10 SLC52A3 variants were not previously reported. These novel mutations were: Glu76Lys, Ser128Leu, Ala288Val and Pro363Leu in SLC52A2 and, Ile20Leu, Val118Met, Thr124Asn, Thr135Ala, Arg212Cys, Phe224Cys, Tyr276X, Gly418Asp and Trp431X in SLC52A3. Mitochondrial respiratory complex I and complex II activity and mitochondrial membrane potential were decreased in SLC52A2 mutation patients and carrier fibroblasts as a possible consequence of a deficit in riboflavin, FAD and FMN status. Preliminary data will be presented regarding the effect of a knockdown on aspects of mitochondrial function in *Drosophila*.
Conclusions: Overall our findings confirm the pathogenetic role of SLC52A2 and SLC52A3 in BVVL, and thus have important clinical and therapeutic implications.

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Whole exome sequencing – a Pandora's box?

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Background: Whole-exome sequencing (WES) is a recently developed technique in genetics research that attempts to identify causative mutations in complex, undiagnosed genetic conditions. Causative mutations are usually identified after filtering the hundreds of variants on WES from an individual's DNA selected by the phenotype.
Aims: Using WES, we aimed to identify the causative mutation in a patient with a slowly progressive distal motor neuropathy with extrapyramidal signs.
Patient and Methods: A 71-year-old man presented with back pain, hand weakness and a neuropathic gait in his forties. He subsequently developed cerebellar and extrapyramidal signs such as dysdiadochokinesis, broken ocular pursuit and torticollis. We carried out basic blood, neurophysiological and radiological investigations, as well as a muscle biopsy and a genetic screen for the more common genetic diseases matching the phenotype. We performed WES of the patient's genomic DNA using Illumina TruseqTM 62Mb exome capture and filtered the results by minor allele frequency and predicted pathogenicity using online prediction tools (MutationTaster, SIFT, Polyphen2, A-GVGD, and LRT). Potential pathogenic variants were confirmed by Sanger sequencing.
Results: We investigated a patient with a slowly progressive chronic axonal distal motor neuropathy and extrapyramidal syndrome using WES, in whom common genetic mutations had been excluded, and in whose family no affected members were available to test for segregation. Variant filtering identified potentially deleterious mutations in three known disease genes: *DCTN1*, *KIF5A* and *NEFH*, which have been all associated similar clinical presentations of amyotrophic lateral sclerosis, Parkinsonism and/or hereditary spastic paraplegia. However, no single variant could be definitely identified as being causative.
Conclusions: This case highlights the difficulties and pitfalls of applying WES in patients with complex neurological diseases and serves as a reminder of the importance of clinical skills for shortlisting candidate mutations in the search for the causative one.

P92

Cellular pathomechanisms of hereditary sensory and autonomic neuropathy type 1 (HSAN-1) in primary motor neurons

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Background: HSAN-1 is a form of CMT that is caused by missense mutations in the *SPTLC1/ SPTLC2* genes, which code for two subunits of the enzyme serine palmitoyltransferase (SPT). SPT catalyzes the first and rate-limiting step of de novo sphingolipid synthesis. It has been proposed that mutations in SPT result in a change in enzyme substrate specificity which results in the production of atypical sphinganines- deoxysphinganine (DSp) and deoxymethylsphinganine (DMSp), rather than the normal enzyme product, sphinganine (Sp). Accumulation of DSp and DMSp are proposed to cause neurodegeneration, as seen in HSAN-1 patients.
Aims and methods: We aim to characterize DSp and DMSp-mediated neurotoxicity in primary mouse motor neurons, by assessing cell survival and neurite outgrowth in primary motor neurons following exposure to different doses of Sp, DSp or DMSp. We are also investigating the potential mechanisms that underlie DSp/DMSp neurotoxicity, by characterizing mitochondrial function and changes in calcium concentration.
Results: Our results show that the abnormal enzyme products DSp and DMSp have a dose-dependent, neurotoxic effect in primary motor neurons. These abnormal sphingolipids not only cause cell death but also result in decreased neurite outgrowth. In addition, motor neurons treated with DSp and DMSp also display significant mitochondrial abnormalities as well as abnormal intracellular calcium levels.
Conclusion: These results show that the aberrant sphinganines have a clear, dose-dependent neurotoxic effect in primary motor neurons.

P93

Plasma neurofilament heavy chain levels in Charcot-Marie-Tooth disease

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Background: The negative trials of vitamin C in CMT1A have highlighted the lack of a sensitive outcome measure in Charcot-Marie-Tooth disease (CMT). Neurofilaments are the most abundant neuronal cytoskeletal protein and are expressed at low levels in non-neuronal tissue. The concentration of neurofilaments in the blood is therefore likely to reflect axonal breakdown and as denervation is the principal pathological process in CMT, changes in plasma NFH may reflect the severity of CMT.
Aims: To evaluate the use of plasma NFH as a potential biomarker for monitoring disease progression in CMT.
Methods: Blood samples were collected from healthy volunteers and patients with CMT over a 2-year period. Only patients with genetically confirmed CMT were included in the study. The disease severity was measured using the CMT Examination Score (CMTES). An in-house sandwich ELISA was used to measure plasma NFH levels.
Results: Baseline plasma samples were collected from 90 CMT patients and 79 control participants. For longitudinal analysis, yearly blood samples for plasma analysis were obtained from 19 of 90 patients with CMT and 10 of 79 control participants. A comparison of plasma NFH concentrations between CMT patients and healthy volunteers did not reveal a significant difference between the two groups ($p=0.449$). Unexpectedly, there was a trend towards a reduction in NFH levels with increasing disease severity as defined by the CMTES (Pearson's Rank -0.173, $p=0.104$). There was no significant difference in plasma NFH levels in the CMT group over 1 year (mean difference = -0.02, $p=0.98$). This is despite a significant mean increase in the CMTES of 1 point for all forms of CMT ($p=0.01$).
Conclusions: Plasma NFH levels are not altered in patients with CMT and do not increase with disease severity suggesting that plasma NFH is not a suitable biomarker of disease activity in CMT.

P94

Investigating CMT-2 pathology in patient fibroblasts: a morphological and functional study.

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Background: CMT-2 is a clinically and genetically heterogeneous group of peripheral neuropathies. To date, mutations in more than 40 genes have been described that cause CMT2. These CMT-2 associated genes can be functionally grouped into 4 categories or proteins: i) cytoskeletal, ii) mitochondrial, iii) endosomal trafficking and iv) microtubule- associated motor proteins. Although the mechanisms by which mutations in these diverse groups of proteins cause a common disease remain unclear, recent reports suggest that disturbances in axonal transport, mitochondrial function and cytoskeletal assembly may play a role in the pathology of at least some forms of CMT-2.
Aims: In this study we aimed to investigate the underlying pathomechanisms of CMT-2 by studying histological and functional abnormalities in cultured fibroblasts derived from CMT-2 patients.
Methods: Cytoskeletal abnormalities were investigated using immunohistochemistry and Western blots, and mitochondrial function was examined by live cell imaging using fluorescent confocal microscopy. Fibroblasts from patients with a number of disease causing mutations were examined, including mutations in NFL, MFN2 and HSPB1, in addition to fibroblasts from age-matched healthy controls.
Results: Patient fibroblast cells from all mutations were found to have normal cytoskeletal morphology and displayed normal microtubular and actin filament staining patterns. The effects of CMT-2 mutations on mitochondrial function is currently under investigation.
Conclusions: Although the use of patient fibroblasts cells has limitations as a cell culture model of CMT-2, it can be used to reveal and exclude basic underlying cellular abnormalities.

P95

Exploring the causes of falls and balance impairments in people with neuropathy

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Background and Aim: Falls are commonly reported by people with Charcot-Marie-Tooth disease (CMT). To successfully manage the problem of falls there needs to be greater understanding of the causes of balance and gait impairment. This study explores balance performance and falls frequency in people with CMT, to ascertain the relationship with their clinical presentation.
Methods: People with CMT and matched healthy controls were recruited. Quantitative lower limb muscle testing, sensory measures, disease severity (CMT examination Score –CMTES), functional balance scales (Tinetti Balance Scale, Berg Balance score, Bruininks Osteretsky Test –BOT) and self-reported balance and walking questionnaires (Modified Falls Self Efficacy scale -mFSE, Walk-12) were administered. Falls events were recorded over 6 months using weekly postcards for participants with CMT to record falls or near falls. Kinematic and kinetic analysis of walking and balance was performed in all subjects. Group comparisons were performed using unpaired t-tests and Mann-Whitney U tests, depending on data type. Correlations were investigated using Pearson's and Spearman's tests. Significance was set at $p<0.01$ to account for multiple comparisons.
Results: *(1) Group comparison:* To date, 12 of the target 30 people with neuropathy have been recruited and 16 of 30 control subjects. Kinematic and kinetic analysis of balance and gait are yet to be analysed. Early results demonstrate significant worse functional balance performance for people with CMT (BOT: $z = -3.67$, $p<0.01$; Berg: $z = -3.73$, $p<0.01$; Tinetti: $z = -3.73$, $p<0.01$). Self-reported balance confidence and walking performance were also worse in people with CMT (mFSE: $z = -4.18$, $p<0.01$; Walk-12: $z = -4.04$, $p<0.01$). People with CMT were significantly weaker in the distal muscles, had higher vibration thresholds and reduced light touch sensation. *(2)*

Balance performance: People with CMT demonstrated strong negative correlations between balance performance measured by the BOT and Berg Balance Scale versus CMTES (BOT: p<0.01; Berg: -p<0.01), vibration threshold at the malleoli (Berg: p<0.01), tibia (BOT: p<0.01; Berg: p<0.01) and dorsiflexor strength (BOT: p<0.01; Berg: p<0.01). *(3) Falls frequency:* People with CMT fell an average of 5±6 times in 6 months and reported 7±12 near falls. Falls frequency was most closely, though not significantly, related to the BOT score (r = -0.68, p<0.05) for functional balance. Vibration disappearance threshold at the malleolus and tibia were the only impairment measures related to falls frequency (p<0.01). **Conclusions:** This early analysis indicates that people with CMT have greater distal weakness and sensory impairment than healthy controls that relate to worse functional balance performance. The rate of actual falls, however, relates to sensory impairment only. This may be because of poorer perception of balance threat leads to a delay in a corrective motor response.

Glycosylation Disorders

‡P96

Mutations in GMPPB cause congenital myasthenic syndrome and bridge myasthenic disorders with dystroglycanopathies

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Congenital myasthenic syndromes (CMS) are a group of inherited disorders that arise from impaired signal transmission at the neuromuscular junction (NMJ). Mutations in 20 genes are known to lead to the onset of these conditions. Four of these, ALG2, ALG14, DPAGT1 and GFPT1, are involved in glycosylation. Here we identify a fifth glycosylation gene - GMPPB, where mutations cause CMS. We identified recessive mutations in 7 cases from 5 kinships defined as CMS using decrement on repetitive nerve stimulation on electromyography (EMG). The mutations were present through the length of the GMPPB, and segregation, in silico analysis and western blots were used to determine pathogenicity. GMPPB-CMS cases show similar clinical features to other glycosylation-CMS subtypes, with variable weakness of proximal limb muscle groups while facial and eye muscles are largely spared. However, patients with GMPPB-CMS had more prominent myopathic features that were detectable on muscle biopsies, EMG, muscle MRI, and through elevated serum creatine kinase levels. Mutations in GMPPB have recently been reported to lead to the onset of Muscular Dystrophy Dystroglycanopathy (MDDG). Analysis of four additional GMPPB-MDDG cases by EMG found that a defective NMJ component is not always

present. Thus, we find mutations in GMPPB can lead to a wide spectrum of clinical features where deficit in neuromuscular transmission is the major component in a subset of cases. Clinical recognition of GMPPB-CMS may be complicated by the presence of myopathic features, but correct diagnosis is important because affected individuals can respond to appropriate treatments.

P97

A phase II pilot study to explore treatment with sodium valproate in adults with McArdle disease (glycogen storage disorder type V, GSDV)

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Background: Currently, there is no satisfactory treatment for McArdle disease. Sodium valproate is one of a group of drugs known as histone deacetylase inhibitors (HDACIs) that can affect gene expression by acetylating lysine residues, which in turn has a direct effect on chromatin. A recent clinical trial of the drug in McArdle sheep that were given sodium valproate showed the presence of phosphorylase positive muscle fibres.
Aims: The aim of this pilot study is to determine the feasibility of performing a clinical trial of sodium valproate in people with McArdle disease.
Methods: 15 subjects will receive sodium valproate modified release 20mg/kg/day (maximum dose 2.0g/day) administered orally once daily for six months. Outcome measurements include cycle ergometry (rating of perceived exertion, oxygen consumption and the respiratory quotient, serum lactate and ammonia levels, maximum heart rate and workload), the number of phosphorylase positive fibres on muscle biopsy pre and post treatment, maximum walking distance measured by the 12 minute walk test, forearm exercise test, blood laboratory parameters, quality of life questionnaire, symptom diary and side effect diary.
Results: We expected an improvement in functional capacity in patients treated with sodium valproate in association with an increase in phosphorylase expression in muscle fibres.
Conclusion: This pilot study might be the initial step in exploring a novel treatment option for patients with McArdle disease.
Funder: Muscular Dystrophy Campaign

P98

FKRP is not required for glycosylation of α- dystroglycan in the heart during development

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Background: The secondary dystroglycanopathies are a heterogeneous group of muscular dystrophies characterised by the hypoglycosylation of α- dystroglycan. Cardiomyopathy (dilated, or less commonly, hypertrophic), systolic dysfunction and/or myocardial fibrosis have been identified in a number of dystroglycanopathies, including Fukuyama-type congenital muscular dystrophy (FCMD), CMD Type 1C (MDC1C) and LGMD2I associated with mutations in Fukutin or Fukutin related protein (FKRP). Studies have demonstrated that more than half of patients with LGMD2I have some degree of cardiac involvement, and in a proportion of patients, cardiomyopathy may be the presenting clinical complaint. Mice with a deficiency of FKRP ($FKRP^{KD}$) show a marked reduction in α-dystroglycan glycosylation (as indicated by immunolabelling with IIH6) and as such are a model for the secondary dystroglycanopathies.

Aims: In view of the clinical significance of the heart the present study sought to examine α-dystroglycan glycosylation during development of the heart in the $FKRP^{KD}$ mice.
Results: Here we show that contrary to expectation the hearts of $FKRP^{KD}$ mouse embryos at E12.5 and E15.5 labelled with IIH6 despite a failure to express glycosylated α- dystroglycan elsewhere. Furthermore using a Fkrp reporter mouse, in which the expression of FKRP is indicated by the expression of EGFP, we show that Fkrp is not expressed in the cardiomyocytes until P0.
Conclusions: In summary these findings indicate that Fkrp is not required for expression of the IIH6 epitope during the early stages of heart development. This may indicate an alternative pathway for glycosylation of α-dystroglycan during development.
This work is funded by the Muscular Dystrophy Association (MDA).

P99

Dystroglycan glycosylation and secondary myogenesis in FKRP Deficient ($FKRP^{KD}$) Mice.

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Background: The defective glycosylation of α-dystroglycan is associated with a group of muscular dystrophies collectively referred to as the secondary dystroglycanopathies. FKRP mutations are one of the most common underlying causes of dystroglycanopathy in the UK and are associated with a wide spectrum of disease.
Aims: In view of the pivotal role of α-dystroglycan in basement membrane deposition we sought to determine if myogenesis was perturbed in a mouse model of FKRP related dystrophy. $FKRP^{KD}$ mice showed a marked reduction in α-dystroglycan glycosylation and laminin binding relative to wild type by embryonic day 15.5 (E15.5). Whilst primary myogenesis was not altered, the number of Pax7 positive cells in the $FKRP^{KD}$ tibialis anterior but not the extensor digitorum longus was significantly reduced. At E15.5 cluster size (primary and secondary/tertiary myotubes enclosed within a single basement membrane) showed a significant decrease in the $FKRP^{KD}$ tibialis anterior relative to wild type suggesting a defect in the ability to form secondary and tertiary myotubes. Myoblasts isolated from the limb muscle of these mice at E15.5 showed a marked reduction in their ability to form myotubes *in vitro* lending support to this hypothesis.
Conclusions: Alterations in basement membrane deposition are a feature of $FKRP^{KD}$ muscle from the early stages of secondary myotube formation and in the tibialis anterior muscle this is associated with a reduction in Pax 7 positive satellite cells thereby potentially comprising subsequent muscle growth and/or the ability of the muscle to regenerate.
This work is funded by a studentship from the Muscular Dystrophy Campaign.

P100

Cajal-Retzius cell mislocalisation correlates with the severity of structural brain defects in mouse models of dystroglycanopathy.

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Background: The secondary dystroglycanopathies are heterogeneous neuromuscular disorders characterised by the hypoglycosylation of α-dystroglycan, and are associated with mutations in at least 17 genes.
Aims: To undertake a comparison of the brain phenotype of three dystroglycanopathy mouse models in order to determine if there was any evidence of gene-specific aspects and to what extent they recapitulate the human disease.
Results: We show that $FKRP^{KD}$ mice exhibit the most severe brain phenotype, with more extensive disruption to the glia limitans than $Pomgnt1_{null}$ or $Large^{myd}$ mice. Furthermore mislocalisation of Cajal-Retzius cells correlates with the severity of the brain phenotype, and many aspects of the pathology observed in the mice recapitulate the situation in human patients.

Conclusions: Cajal-Retzius cells typically respond to cues originating from the meninges, our findings suggest that whilst cortical defects in Large^{myd} mice are consistent with a disturbance of the radial glial scaffold, the more severe phenotype observed in FKRP^{KD} and Pomgnt1$_{null}$ mice may imply additional defects in the meninges. Overall these observations implicate gene specific differences in the pathogenesis of brain lesions in this group of disorders.

IBM

‡P101

Using whole-exome sequencing to identify mutations of *SQSTM1* and *VCP* in inclusion body myositis

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Background: Sporadic inclusion body myositis (sIBM) is the most common acquired myopathy in people aged over 45 years. It is a complex disease and although the primary pathogenic mechanism is unknown, there is evidence that multiple factors, including genetics, are implicated in the disease process.
Aim: As part of the International IBM Consortium Genetics Study, we aim to perform whole-exome sequencing in 200 IBM patients to explore genetic factors associated with IBM.
Methods: We have recruited 164 patients with sIBM, and two siblings with a form of hereditary IBM (hIBM) so far. Whole-exome sequencing has been performed in all the cases. The filtering strategy is for rare (frequency <0.1%) non-synonymous variants in candidate genes, including known inherited IBM genes, genes associated with inflammation and neurodegenerative diseases. All candidate variants in genes of interest were confirmed by Sanger sequencing.
Results: Four rare variants in the sequestosome 1 (*SQSTM1)* gene and three rare variants in the valosin containing protein (*VCP)* gene were found in our sIBM cohort, and a known pathogenic mutation in the *VCP* gene was found in the two hIBM siblings. The sIBM variants are likely to be *de-novo* as there was no family history. Among them, the p.P392L mutation in *SQSTM1* is the most frequent *SQSTM1* mutation reported in Paget's disease of bone (PDB) and is also associated with amyotrophic lateral sclerosis (ALS). Two of the three *VCP* mutations found in sIBM cases have also been reported in an inherited form of IBM – IBM with Paget's disease and frontotemporal dementia.
Conclusion: This is the first time that mutations in *SQSTM1* and *VCP* have been found in sIBM. Although unproven as yet they are likely *de-novo*. This suggests that *SQSTM1* and *VCP* might be genetic susceptibility factors for IBM. The occurrence of the same mutation in IBM, PDB and ALS may indicate a common pathogenic link resulting in impaired autophagy-lysosome pathways, causing further dysregulation of protein homeostasis.

‡P102

Investigating the effects of pharmacological up-regulation of the heat shock response in a transgenic mouse model of inclusion body myopathy

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Sporadic inclusion body myositis (sIBM) is the most common acquired muscle disease affecting adults over the age of 50. The precise cause of sIBM remains unknown, but the disease is characterised by both inflammatory and degenerative features. Trials of anti-inflammatory agents have all been unsuccessful and to date there is no effective disease-modifying treatment for sIBM

Protein mishandling in cells leads to the accumulation and aggregation of proteins, and this is characteristic of IBM pathology. Previous work in our lab has demonstrated that co-induction of the cytoprotective heat shock response (HSR) with Arimoclomol ameliorates IBM-like pathology in cultured muscle cells by improving protein handling.

To take this forward into an in vivo pre-clinical trial, we acquired a transgenic mouse model of multisystem proteinopathy (MSP) caused by a mutation in the valosin-containing protein (VCP) gene. This model recapitulates many of the key features of sIBM in muscle. In this study, we examined changes in the muscle of the mutant (mVCP) mice to gain insight into the underlying pathomechanisms of the disease. By characterising the pathological features of muscle in this mouse model we hoped to obtain outcome measures to assess the therapeutic effects of a pre-clinical trial of Arimoclomol. In addition protein degradation pathways were also assessed and the effects of Arimoclomol on these mechanisms were investigated. The methods used in this study include immunohistochemistry, western blot analysis, biochemistry and imaging techniques.

Histological examination showed evidence of key IBM-like characteristics in the muscle of mVCP mice, including TDP-43 mislocalisation, ubiquitin-positive inclusions and an increased number of centralised nuclei. Treatment with Arimoclomol was found to significantly attenuate these pathogenic features in muscle *in vivo*.

These *in vivo* findings build upon our *in vitro* data and together suggest that Arimoclomol may be a potential therapeutic agent for the treatment of sIBM.

P103

Does NRLP3 inflammasome activation take part in muscle inflammation?

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Background: There is emerging evidence that myositis is a complex disease with multiple pathogenic pathways simultaneously contributing to muscle damage and weakness. Among these the most prominent are the innate, adaptive and metabolic pathways. Innate immune pathways include activation of the molecular platform known as the NRLP3 inflammasome and in consequence secretion of pro-inflammatory cytokines such as IL-1B and IL-18 link the adaptive and metabolic arms of the disease process.

Aims: The study investigates NRLP3 inflammasome behaviour in inflammatory muscle diseases such as inclusion body myositis and dysferlin deficiency.

Methods: The immunochemistry using anti-Caspase1, anti-ASC and anti NALP3 antibodies have been used to access and stain muscle tissue from patients with inclusion body myositis.

Caspase 1, ASC and NALP3 protein are part of NARLP3 inflammasome platform.

Imunochemistry was performed to stain the colon tissue samples from patients with diverticular disease and used as a positive control.

Results and Conclusions: The preliminary data suggests that tissue samples from diverticular disease can be considered as a positive control for further investigation of the NARLP3 inflammasome activation in inclusion body myositis

P104

Mutational spectrum and phenotypic variability of VCP related neurological disease in the UK

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Background: IBMPFD (OMIM number 167320), or hereditary inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia is a rare autosomal dominant disorder due to mutations in the valosin-containing protein gene (*VCP*). VCP is a ubiquitous protein member of the AAA+ (ATP-binding proteins) protein family involved in multiple cellular functions.
Objective: To describe the clinical, pathological, and genetic findings of 42 individuals from 21 families with VCP mutations diagnosed since 2009 in the UK.
Methods: Patients with genetically confirmed IBMPFD were identified at the Newcastle MRC Neuromuscular Centre and the clinical details, muscle biopsy findings and muscle MRI data were collected retrospectively.
Results: We estimate a point prevalence of IBMPFD of 0.066/100 000 for the UK population. Muscle weakness was the leading symptom in 92.3% of the patients, either with a limb-girdle pattern and/or distal weakness. The mean age at onset was 42.8 years and the mean time to loss of ambulation 13.37 years. Parkinson's disease, bladder, anal, and erectile dysfunction were additional features in a minority of patients. Two patients required assisted ventilation and four patients developed cardiomyopathy. Dementia or mild cognitive impairment was observed in 48.2% and Paget disease of the bone was present in 20.5% patients. All muscle biopsies showed myopathic changes, 61% had rimmed vacuoles and 33.3% small inflammatory infiltrates. We have identified four previously described missense mutations (p.R155C, p.R155H, p.R191Q, and p.R93C) and 2 novel mutations (p.G202W and p.A439G).
Conclusions: IBMPFD is rare neuromuscular condition, probably under diagnosed due to the variability in phenotypes frequently showing multisystem involvement. We suggest VCP-related disease as a term that encompasses the muscle, bone, and central nervous system manifestations. Larger cohorts are needed in order to produce clear guidelines for the diagnosis and management of patients with VCP-related diseases.

Muscle Satellite Cells and IPS Cells

‡P105

Towards a genomic integration-free, iPS cell and human artificial chromosome-based therapy for Duchenne muscular dystrophy

Authors

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Background: Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene and affects skeletal muscles, resulting in disability and premature death. There is no cure for DMD, but several experimental therapies are under investigation. Among these, gene and cell therapy is complex, with dystrophin being the largest human gene (2.4Mb) and skeletal muscle the most abundant human tissue. The combination of both Human Artificial Chromosome-based gene correction and iPS cell-mediated production of transplantable myogenic cells can overcome two of the main obstacles for gene and cell therapy of DMD, such as the large size of dystrophin and the limited availability of large number of cells. We have pioneered HAC transfer and derivation of myogenic cells from human iPS cells; however, safer strategies to translate these technologies into future clinical protocols still need to be developed.

Aims: Here we present aims and preliminary results of a new project aimed at generating transplantable, clinically-relevant DMD iPS cell-derived myogenic progenitors genetically corrected with HACs containing the entire human dystrophin locus.

Methods: DMD iPS cells are generated with non-integrating strategies, including Sendai viruses. The integration-free iPS cells are then differentiated into myogenic progenitors using also integration-free myogenesis regulators, such as MyoD.

Results: Preliminary results indicate that myogenic cells similar to skeletal muscle pericytes/mesoangioblasts can be generated from DMD iPS cells genetically corrected with HACs. A novel HAC capable to induce MyoD-mediated differentiation without genomic integration was also developed.

Conclusion: The generation of HAC-corrected DMD iPS cell-derived myogenic cells demonstrates the feasibility of a new, safer integration-free approach for ex vivo gene therapy of DMD.

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Overcoming challenges in muscular dystrophy research: genome editing tools for targeted gene correction in patient-specific iPSC.

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Background: Our research focuses on the study of allelic mutations in the putative glycosyltransferase *FKRP* gene, which cause a wide range of muscular dystrophy (MD). The most severe forms are characterized by an early onset of the MD phenotype associated with central nervous system (CNS) defects, but little is known about the molecular and cellular mechanisms underlying the CNS involvement. We generated patient-specific induced pluripotent stem cells (iPSC) carrying a FKRP(A455D) mutation associated with neurological defects. These iPSC can be differentiated to neurons for studying CNS involvement in MD. One major challenge of working with iPSC is the lack of an appropriate isogenic control, which minimizes the variability between different genetic backgrounds.
Aims: In order to address this problem, we aim to develop an isogenic pair of control and patient-specific iPSC and use it as a model to study FKRP-deficient MD.
Methods: Site-specific transcription activator-like effector nucleases (TALEN) and CRISPR (clustered regulatory interspaced short palindromic repeat) based RNA-guided DNA nucleases, combined with *piggy*Bac transposon technology, were used to correct the FKRP(A455D) mutation in patient-specific iPSC.
Results: Upon TALEN- or CRISPR-mediated homologous recombination, a targeting donor vector with the corrected sequence could be successfully integrated into the genomic DNA of patient-specific iPSC. PCR based genotyping and sequencing confirmed the achievement of a clean biallelic correction of the FKRP(A455D) mutation.
Conclusion: TALEN or CRISPR in combination with *piggy*Bac technology are versatile tools, which allow precise modification of the mammalian genome at single base-pair levels and allow mutation correction in patient-specific iPSC. This can be used as an isogenic control for disease modelling. It is tempting to speculate that in the future, this isogenic iPSC MD model could be exploited for genetic and molecular screens aiming to identify therapeutic targets.

P107
Developing 3D scaffolds to support myogenesis
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Background: Skeletal muscle can repair/regenerate efficiently due to resident muscle stem cells called satellite cells. However, this process is ineffective for restoring significant muscle loss following trauma, such as after road traffic accidents or tumour removal. Intramuscular injection of myogenic cells can improve muscle regeneration, but this approach is inefficient. One strategy to facilitate larger scale muscle regeneration or replacement, is transplantation of myogenic cells together with a biocompatible scaffold to provide a supportive environment for both donor and host cells.
Aims: To test 3D scaffolds for their ability to support skeletal myogenesis.
Methods: The ability of primary murine satellite cells on isolated myofibres to proliferate and form myotubes in several 3D biocompatible scaffolds was analysed.
Results: Three biomaterials were used to create 3D scaffolds: collagen gel, polyethylene glycol-fibrinogen hydrogel (PEG-FN) and fibrinogen-based gel. Expanded primary satellite cells embedded in these 3D scaffolds generally survived but did not differentiate well. However, freshly isolated myofibres with their associated satellite cells were able to produce many myoblasts that proliferated and differentiated into large multinucleated contractile myotubes. By generating tension across the biomaterial scaffold, it was possible to orientate the myotubes and so create directed contraction.
Conclusion: Freshly isolated satellite cells associated with a myofibre differentiate more efficiently than expanded satellite cells. Importantly, application of tension across the 3D scaffold facilitated aligned myotube formation, so contraction was directed along the long axis of the gel. Why myotube formation in 3D Scaffolds was enhanced by the presence of muscle fibres is unclear, but could be trophic factors and/or associated fibroblasts.

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Databases, Diagnostics and Clinical Practice

P108

Cardiac disease is under recognised in patients with HMERF

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Background: Hereditary myopathy with early respiratory failure (HMERF) is an autosomal dominant disorder occurring due to the c.951434T>C; (p.Cys31712Arg) missense mutation in the *TTN* gene[1]. HMERF is characterised by adult onset of distal and/or proximal muscle weakness in association with early respiratory muscle weakness which may require non-invasive ventilation. Cardiac complications of *TTN* missense mutations are not reported[1-4], although truncating *TTN* mutations are implicated in various cardiomyopathies[5,6]. We questioned this given the similarities between cardiac and skeletal *TTN* splice-isoforms.

Aims: To identify the presence of cardiac disease in HMERF.

Methods: We reviewed clinical notes and arranged a routine echocardiogram for all patients known to our centre with HMERF.

Results: We identified 20 patients from 8 families. Two had confirmed recurrent supraventricular tachycardia (SVT) treated with bisoprolol, and one underwent cardiac ablation. One further patient complained of palpitations but no specific diagnosis was reached.

ECHO

19 patients underwent echocardiogram.

1. Left Ventricular Function
   Two patients were under cardiology review: one had a mildly progressive reduction in left ventricular ejection fraction (LVEF), and one had LVEF of 30% in 2008, improving to 55% in 2014 following introduction of medical management. In all remaining patients, LVEF was >55%.

2. Diastolic Function
   Four patients had an E/E' ratio between 8 and 15; possibly indicating mild diastolic dysfunction. One took Lisinopril for hypertension, the remainder had no cardiac history. None had other ECHO abnormalities.

Conclusions: Cardiac involvement appears to be under-reported. SVT is over-represented (p=0.002). LV dysfunction may respond to cardio-protective treatment.

No significant relationship between ECHO abnormalities and respiratory involvement (p=0.51); or use of NIV (p=1) was identified.

Cardiac MRI will determine relevance of diastolic findings.

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P109

SMA REACH UK one year on: the evolution of robust functional outcome measures for spinal muscular atrophy type 2 & 3

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Background: Increasing momentum in the field of Spinal Muscular Atrophy (SMA) has resulted in potentially promising experimental therapies entering clinical trials. Robust functional outcome measures are required to demonstrate efficacy of experimental therapies and for their regulatory approval.
SMA REACH UK has been working collaboratively with the Italian SMA Network and the PNCR Network USA to develop and refine functional outcome measures for use in SMA, to meet requirements of regulatory authorities.
Aims: To develop psychometrically robust functional outcome measures for use in type 2 and 3 SMA.
Methods: SMA REACH UK hosted 2 international workshops (2013, 2014), national meetings and numerous teleconferences, where expert physicians and physiotherapists meticulously discussed functional outcome measures for SMA. Utilising item response theory, expert opinion and experience from clinical trials, the Hammersmith Functional Motor Scale Expanded and Upper Limb Module for SMA were used as the foundation for the development of new scales. Two revised scales have been prospectively piloted and further refined over the last twelve months.
Results: The Revised Hammersmith Scale for SMA (RHS): Following three iterative revisions and 122 assessments, we outline the final proforma, its development, and Rasch analysis. The RHS is undergoing further responsiveness and psychometric testing in a wider international cohort to endorse its content and the cohesiveness of items.
The Revised Upper Limb Module for SMA (RULM) (led by the Italian Network): 95 assessments are available for this revised scale. Psychometric analysis has shown good fit of all items and suitable targeting; however a ceiling effect still exists for the stronger type 3 patients.
Conclusion: Initial findings of the RHS and RULM indicate that they will make promising alternatives to the original scales whilst also meeting the rigorous psychometric requirements of regulatory authorities.

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UK Patient Registry for Facioscapulohumeral Muscular Dystrophy (FSHD)

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Background: The UK Facioscapulohumeral Dystrophy (FSHD) Registry is funded by the Muscular Dystrophy Campaign and supported by the TREAT-NMD Alliance. This online patient driven registry aims to facilitate and accelerate planning and recruitment of clinical trials. Patient reported outcomes are entered into a secure online portal combined with verified genetic details.

Aims and Methods: Core clinical and genetic information has been collected through the registry with additional data about pain (MPQ-SF) and quality of life (INQoL). Here we analyse this data to describe the characteristics of the 416 FSHD1 patients registered between May 2013 and December 2014.

Results: The current age range of participants is 4 to 83 years old (mean 47.58 +/- 16.5) with an even distribution between genders (216 males, 200 Females). Wheelchair use at least part-time is reported by 166 participants, 76 report some hearing loss (17 under 40 years old) and 37 use a ventilator (at least part-time). The onset of muscle weakness in 46% of cases is reported between 10 and 29 years old (mean 20.45 +/- 15.95). Weakness in the facial muscles occurs significantly earlier than in other areas (mean 18.83 years +/- 15.30). In this cohort it is weakness that has the greatest impact on quality of life, above pain and fatigue, and impacts the ability to carry out daily activities and body image more than other Quality of Life domains (emotions, relationships and independence).

Conclusion: The registry provides a resource to better understand the FSHD population in the UK that can be used to inform future research and develop standards of care. The registry is well placed to be involved in initiatives to harmonise data not only among neuromuscular diseases as part of TREAT-NMD but also across the wider rare disease community through RD-Connect and the aims of IRDiRC.

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GNE myopathy disease monitoring program as a tool in translation research for ultra-rare disease.

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Background: GNE myopathy is an ultra-rare autosomal recessive distal myopathy due to mutations in *GNE*. *GNE* encodes for an enzyme that catalyses the first 2 steps of sialic acid biosynthesis. There is a need to better understand the disease to heighten disease awareness; expand knowledge of the clinical characteristic and optimize patient management.

Aims The aim is to assess the course of GNE myopathy; discover worldwide epidemiology and facilitate further clinical research in the field.

Methods: The GNE disease monitoring program is designed to meet the objectives by following patients over time using a patient registry and a natural history study. The registry is a patient self-reported online database available online to GNE patients worldwide.

Results: By January 2015, 131 registrants were on the registry and 78 (60%) completed questionnaires. Patients residing in Europe represent over 40% of patients, followed by Middle East (21%) and North America (16%). Patients from 20 countries are represented in the registry with 70% coming from: Iran, Italy, USA and UK. Male/female ratio was 0.4 with the mean age 41 years. Ambulation status is preserved in 59% of patients, 17.4% have limited ambulation and 23.5% are non-ambulant. A range of assistive devices is in use with the most common being ankle orthotics. Diagnostic related difficulties observed in GNE myopathy are: limited access to genetic testing; low awareness of the disease; unspecific muscle biopsy presentation due to wrong site of sample collection; absence of clear guidance for patients with single *GNE* mutation; low cost genetic testing of founder mutations only.

Conclusion The international registry is a useful tool for translation research in ultra-rare disease. The GNE myopathy registry was well received by patients and identified a number of diagnostic, country specific issues which need to be addressed in order to improve GNE myopathy management.

P112

Exome sequencing in undiagnosed inherited and sporadic ataxias

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Background: Precise diagnosis of hereditary ataxias is important because it leads to accurate genetic counselling and aids in diagnosis for families or patients with similar phenotypes.
The current diagnostic laboratory approach is stepwise genetic testing, which is expensive and time consuming. Exome sequencing is gradually establishing itself as an excellent tool to elucidate causative variants in various neurological diseases. Targeted next-generation sequencing panels (i.e. ataxia multi-gene panels) could have a limited portfolio of genes included on custom-designed platforms and therefore have a lower diagnostic yield.
Aims: To establish if exome sequencing could be successfully used to identify the genetic cause of neurological disorders
Methods: We used a heterogeneous cohort of patients with suspected inherited ataxia as an example of a neurological disorder and applied whole exome sequencing in order to assess its usage in a clinical setting (Illumina TruSeq™ 62 Mb and HiSeq 2000, 100 bp paired-end reads). After excluding common sporadic and inherited causes, we used a whole exome sequencing approach in 35 affected individuals from 22 randomly selected families of white European descent.
Result: We defined the likely molecular diagnosis in 14 of 22 families (64%) with a total of 11 genes implicated. This revealed *de novo* dominant mutations, validated disease genes previously described in isolated families, and broadened the clinical phenotype of known disease genes. The diagnostic yield was the same in both young and older-onset patients including sporadic cases.
Conclusion: This demonstrated the extreme genetic heterogeneity but shows the impact of exome sequencing in a group of patients notoriously difficult to diagnose genetically.

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Gene panel testing reveals that a high proportion of patients with inherited peripheral neuropathy have autosomal recessive disease.

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Inherited peripheral neuropathy (IPN) is a clinically and genetically heterogeneous group of disorders involving more than 90 genes and various modes of inheritance. We designed a panel assay comprising 56 genes associated with the common and the rare causes of IPN. This is provided as a specialist UK Genetic Testing Network service and was launched as a diagnostic service in July 2013, replacing sequential gene analysis using Sanger sequencing in most cases. Of the 56 genes included in the panel, 26 are associated with an autosomal

recessive form of the condition, and for the vast majority of these genes no diagnostic service was previously available.
We present an overview of 31 patients homozygous or compound heterozygous for pathogenic mutations in 10 recessive genes. These represent one third of our positive diagnosed cases (31/99). 19 patients were referred with CMT1, 8 with CMT2, 1 with dHMN, 1 with HSN and 2 with a complex phenotype. Eight of the 10 genes were not previously available for routine testing in the UK. Case studies will also be presented in detail.
Autosomal recessive IPN is traditionally thought of as a severe, childhood onset form of neuropathy; however in our cohort 19 patients were over 18 years of age at genetic diagnosis, including some cases previously thought to be sporadic. This challenges us to broaden the spectrum of phenotypes associated with known genes, which previously would not have been considered for testing.
Our data shows how powerful a tool gene panel testing is for genetic diagnosis for recessive IPN. It provides a higher diagnostic yield, faster and at a lower cost than sequential single gene screening of the proband, allowing efficient, targeted clinical management and family follow-up.

P114

Next generation sequencing in neuromuscular disease: the Newcastle MRC Muscle Centre effort

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Background: The Newcastle MRC Muscle Centre has a strong clinical and research interest in patients with neuromuscular diseases (NMD). These are genetically heterogeneous conditions and genetic diagnosis cannot always be obtained by conventional genetic testing. We are currently part of three large international collaborations involving WES in patients with phenotypically well characterized and genetically undiagnosed LGMD and other NMDs: NeurOmics, SeqNMD and MyoSeq.
Aims: To identify the causative mutations in undiagnosed NMD patients
Methods: Patients were selected based on clinical and family data. DNA underwent WES in deCODE Genetics (Iceland) and the Broad Institute (USA) as part of NeurOmics and SeqNMD projects. Data was analysed using standard filtering criteria based on population frequency, effect on the protein structure and inheritance model. Variants in known neuromuscular disease genes were investigated first. If no pathogenic variant was identified WES data was analysed for variants in all genes, which were then prioritised by predicted pathogenicity, conservation, biological function and tissue expression. Variants in novel genes were validated in appropriate cell culture and animal models.
Results: We have analysed sequencing data for a total 208 samples, corresponding to 121 families. By means of WES the causative mutation was identified in a ~20% of the families allowing for genetic diagnosis. The majority of causative variants were found in known neuromuscular disease genes and had been previously reported as pathogenic (*CAPN3, TTN, SGCG, COL6A1, COL6A2, MAT3, RBCK1, GMPPB, VCP, AGRN, DOK7, RAPSN,* and *POLG*), whereas others, also in known NMD genes, were novel mutations (*FKTN, RYR1, COLQ, GFPT1, CHRND* and *DPAGT1*). In addition, by means of WES analysis two new NMD genes have been identified (*SCL25A1* and *ALG2*) and the clinical phenotype has been extended for another two genes (*COL12A1* and *PIEZO2*).

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MRC Centre for Neuromuscular Diseases Biobank

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A lack of access to biomaterials from patients with neuromuscular disorders (NMD) has hampered research into and the development of potential therapies for these conditions. The MRC Centre for Neuromuscular Diseases Biobank is a unique repository of human biomaterial aimed at facilitating research of NMD by providing a continuous availability of high quality human biomaterial including muscle and skin cell lines, DNA and also body fluids such as urine, blood, plasma and serum to the scientific community within an ethical framework. The Biobank has been established in close collaboration between Newcastle University and UCL. The Biobank has been actively collecting human biopsy samples since June 2008 from both London and Newcastle using the large neuromuscular patient population seen at both sites. Biopsy samples and blood are predominantly collected from patients with a confirmed or suspected diagnosis of muscular dystrophy, stored and provided to UK and EU partners. In the 7 years since establishment of the centre, the Biobank has surpassed the proposed milestones, benefited a large number of basic, translational research and research projects, supported MRC centre PhD students, resulted in more than 93 high-profile publications, and successfully integrated NMD research in the UK and international networks with more than 200 individual research projects. The Biobank has processed and stored 1335 myoblast, 1424 fibroblast, 12 $CD133^+$ cell, 10 synovial cell, 157 frozen muscle tissue, 22 immortalised fibroblast, 33 immortalised myoblast, 3 frozen skin tissue, 76 peripheral blood lymphocyte, 573 plasma, 837 serum, 163 RNA and 105 urine samples. The samples are associated to clinical data from deep phenotyping of patients making them particularly valuable. Moreover, the Biobank was indispensable in attracting significant subsequent funding from national (MRC and Wellcome Trust) and European (EC, 7th framework program) agencies.

Other

‡P116
Zebrafish as a model system in RNA metabolism deficiencies.
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Background: Impairment of the functionality of the exosome complex, a multi-subunit protein involved in gene expression regulation through RNA degradation, is linked to severe infantile neurological disorders. Our group recently showed that mutations in *EXOSC8*, a subunit of the exosome complex, lead to severe infantile overlapping phenotypes of pontocerebellar hypoplasia type 1 (PCH1), hypomyelination and spinal muscular atrophy. In a single patient with spinal muscular atrophy (SMA)-like symptoms a potentially disease-causing mutation was detected in *RBM7*, a co-factor of the exosome complex which binds and carries to the exosome complex non-coding RNAs, the PROMoter uPstream Transcripts (PROMPTs), in order to be degraded. The role of the PROMPTs is not fully understood but they seem to be involved in transcriptional regulation. RBM7 also acts as a splicing factor. In order to elucidate the different role(s) of RBM7 and other sub-units (EXOSC3 and EXOSC8)

of the exosome complex in vertebrate neurodevelopment, we are performing *in vivo* functional studies using zebrafish (*Danio rerio*) as an animal model.
Methods: Functional knock-down of the genes of interest was performed through specific antisense oligonucleotides (morpholino) in wild type and *islet1*:GFP transgenic fish. Antibody staining of control and down-regulated zebrafish was used to visualize defects in neuromuscular junctions and Purkinje cells.
Results and conclusions: We show that knock-down of *rbm7* in zebrafish leads to delayed development, abnormal body shape and impaired hindbrain patterning which specifically affects vagal brachimotor neurons in *rbm7* down-regulated fish while knock-down of *exosc8* and *exosc3*, differentially affects hindbrain development in zebrafish. Purkinje cell development seems to be affected in *exosc3* but not in *rbm7* down-regulated fish at 4.5 dpf. Motor axon branching is impaired at 48 hpf in *exosc3*, *exosc8* and *rbm7* down-regulated fish. Our results from the zebrafish experiments could provide an explanation for the different disease presentations in patients with mutations in exosome different components.

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Introducing a specialized Masters Course in Neuromuscular Diseases: educating the next generation of scientists, clinicians and professionals allied to medicine at UCL Institute of Neurology

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In order to enhance the training opportunities available in the UK in the field of Neuromuscular Disease, we have designed a new Masters programme that will be run at UCL Institute of Neurology.
This Masters programme aims to equip a wide range of professionals, from clinicians through PAMS and basic scientists with the fundamental principles, biology and latest research and technical advances in the field of neuromuscular disease. The programme builds on the extensive educational expertise present at the UCL Institute of Neurology. The Course aims to train a wide range of specialists, from basic scientists to clinicians, as well as professionals allied to medicine, including nurses and physiotherapists. The Course will take the form of taught lectures and tutorials as well as practicals, workshops and Master Classes. In addition, each student will be expected to undertake a research project under the supervision of world class, leading clinical experts and basic scientists in the field of neuromuscular disease.
The Course has been developed following research into the educational requirements of both professional organizations and professionals themselves. We undertook a survey using the UCL Opinio Survey Platform, which was circulated via the MRC Centre for Neuromuscular Diseases. We received an excellent response, which was used in the course design. The Course shares some modules with other existing programmes within UCL, including the Institute of Child Health, with plans for collaboration with the Neuromuscular Team in Newcastle. The diverse student intake will be supported by tailored Tutoring sessions.
This new Neuromuscular MSc Course is due to start in September 2015 and is now open to applications.

P118

Static mechanical stimulation improves myogenic differentiation in a novel bioreactor-based 3D culture model

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Background: So far, current surgical approaches for the treatment of muscle injury give less than optimal results, emphasizing the need for engineered muscle tissue. We have developed a bioreactor-based 3D *in vitro* biomimetic culture system that allows for mechanical stimulation of myoblasts embedded in a ring shaped fibrin scaffold via magnetic force.
Aim: The aim of the study is to use this platform for patient specific drug screening, muscle trauma research and, on the long term, the generation of functional transplantable muscle constructs.
Methods: Fibrin rings were prepared by injection molding with following final concentrations: 3.2 x 10^6 C2C12 myoblasts encapsulated per ring, 20 mg/ml fibrinogen and 0.625 U/ml thrombin. Rings were either subjected to mechanical strain (6 hours 10% strain per day, started at day 3), kept at 0% strain (CTRL) or allowed to float in media (NEG. CTRL) for 9 days of culture. Myogenic differentiation was assessed by RT-qPCR as well as immunofluorescence confocal microscopy.
Results: C2C12 cells were viable and capable of differentiation within the constructs. RT-qPCR results revealed a significant increase in early-, mid- and late-stage myogenic markers when static strain was applied (Fig. 1). More importantly, analysis of transcription factors MyoD, Myf5 and Myogenin as well as structural genes Desmin and slow skeletal Troponin T showed that myogenic progression within the constructs followed a physiological pattern.

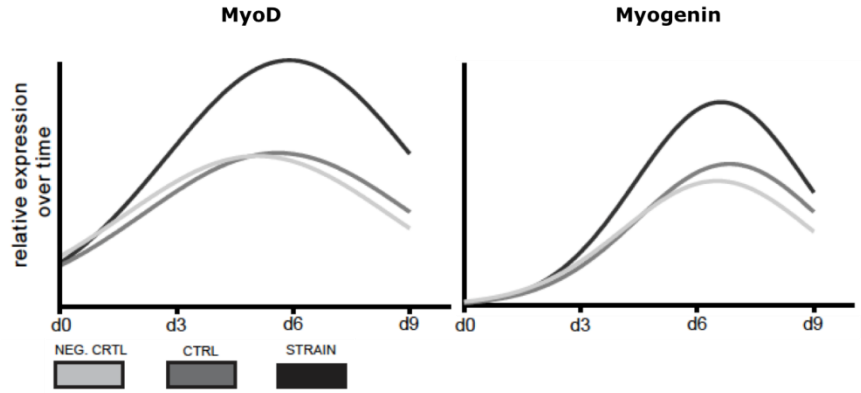

Figure 1: Relative expression levels of MyoD and Myogenin over time.

Furthermore, application of static strain led to parallel myotube alignment and augmented maturity of myocytes (Fig.2).

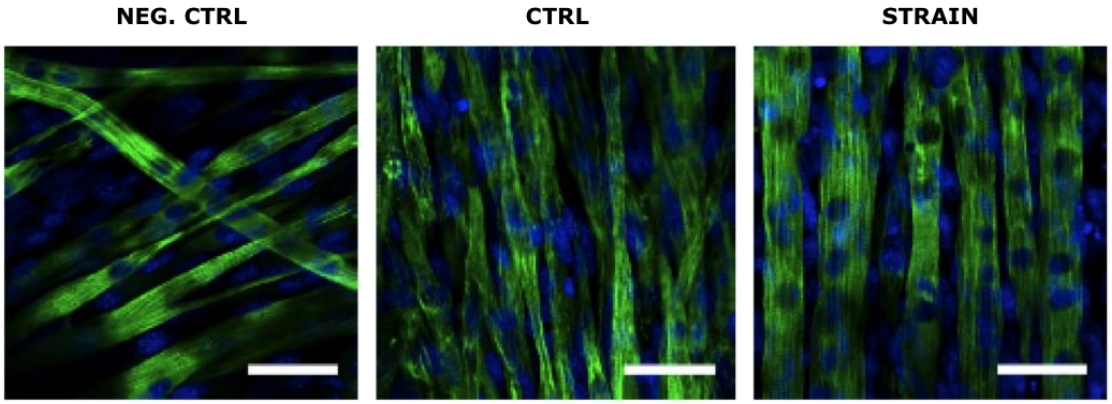

Figure 2 MHC fast (green) and DAPI (blue) staining of C2C12 myotubes after 9 days of culture.

Conclusion: The use of this 3D culture system may provide a powerful tool to study myogenic differentiation, mechanotransduction and muscle physiology or disease. In this respect, optimization of different strain patterns might further increase the degree of myogenic differentiation and functionality, with the long-term goal of providing patients with functional engineered skeletal muscle transplants.

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Red blood cells as therapeutic carrier in monogenic disorders

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Treatments aimed to target skeletal muscles are a big challenge nowadays. As an alternative to conventional medications, which predominately slow down disease progression and relief symptoms in patients, gene therapy opens new promising horizons of a personalised medicine and therapeutic treatments[1]. Pompe´s disease is a metabolic disorder caused by mutations in the acid-alpha-glucosidase (GAA) gene[2]. The deficiency of GAA dramatically impairs skeletal muscle, cardiac and pulmonary function which untimely leads to death before patients turn 1-year old[2]. The aim of this study is to develop a haematopoietic stem cell (HSC) gene therapy to obtain a long-lasting systemic expression of GAA thereby restoring GAA functions in the entire muscular system. To achieve this, we generated a third-generation-lentiviral-vector encoding the GAA gene under the transcriptional control of the ubiquitous mammalian EFS-promoter and β-globin LCR-enhancer which allows overexpression in the erythroid component[3]. In-vitro studies show that the vector increases the expression and activity of GAA in murine-erythroleukemia cells and $CD34^+$-cells differentiated into erythrocyte-like cells in-vitro. As proof of concept in an in-vivo system, HSCs from males of a murine model of Pompe´s disease ($GAA^{-/-}$) were treated ex-vivo with the construct and then transplanted in female recipients. Mice receiving gene-corrected HSCs show high-activity of the enzyme in plasma 3-months post-transplant. Treated and untreated animals will be observed for a period of 5-9 months at the end of which muscular, cardiac and pulmonary functions will be analysed. These preliminary results suggest that red blood cells overexpressing GAA may be a strategic carrier to achieve a systemic and capillary distribution of the defective gene in the wide-spread skeletal muscles throughout the body.

1. Al-Zaidy, S., et al. (2014)
2. Fukuda, T., et al. (2007)
3. Montiel-Equihua, C., et al. (2012)

P120

Investigating the cause of muscle weakness in thin filament myopathies

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Background: Mutations in *NEB*, *ACTA1*, *TPM2*, *TPM3*, *KBTBD13*, *KLHL40* and *KLHL41* lead to thin filament myopathies, such as nemaline myopathy (NM), congenital fiber type disproportion (CFTD) and cap disease (CAP). A hallmark feature of these myopathies is muscle weakness. Thin filament length is a major determinant of force generation.
Aim: Here, we aimed to elucidate whether mutations in *NEB, ACTA1, TPM2, TPM3, KBTBD13, KLHL40 and KLHL41* affect the maximal force generating capacity of muscle fibers. We investigated whether changes in thin filament length contributed to muscle weakness by determining the sarcomere length-dependence of force.
Methods: Biopsies from NM, CFTD, and CAP patients (*n*=43) with mutations in *NEB*, *ACTA1*, *TPM2, TPM3, KBTBD13, KLHL40 or KLHL41* were compared to biopsies from healthy controls (*n*=8). Using permeabilized muscle fibers, maximal active tension (Fmax) was determined at incremental sarcomere lengths (range 2.0–3.5 µm) to obtain the force-sarcomere length relationship. Force data at incremental sarcomere lengths was fitted with a second order polynomial to obtain the optimal sarcomere length for force generation and to calculate thin filament length.
Results: Fmax (in mN/mm^2, mean±SEM)) was significantly lower in biopsies from *NEB* (46±5), *ACTA1* (48±4), *TPM3* (85±10), *KBTBD13* (78±3), *KLHL40* (2.8±0.2) and *KLHL41* (63±4) patients compared to biopsies of controls (129±7). No shift in the force-sarcomere length relationship was observed in *TPM3*, *TPM2, KBTBD13, KLHL40* and *KLHL41* patients. In contrast, *ACTA1* and *NEB* patients showed a leftward shift of the force-sarcomere length relationship indicating shorter thin filaments. In line with this data, muscle fibers from *NEB* patients exhibit significantly shorter optimal sarcomere lengths for force generation.
Conclusion: Our data suggest that mutations in *NEB* and *ACTA1* result in changes in thin filament length. Insights in the mechanisms underlying weakness in patients with thin filament mutations are necessary to improve specific treatment strategies.

P121

Joints contractures, ptosis and external ophthalmoplegia caused by a novel autosomal dominant mutation in *PIEZO2*

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We describe a family presenting with joint stiffness and contractures, spinal rigidity, external ophthalmoplegia and restricted respiratory capacity.
The proband was referred to the muscle clinic at the age of 7 years because of tip toe walking, Achilles tendon contractures, fingers contractures, limited jaw opening and bilateral palpebral ptosis. He had normal cognitive development and a family history of congenital myopathy: his father and his father's sister were born with a similar condition and were diagnosed with "Freeman-Sheldon like syndrome" at birth. Paternal grandmother, a cousin and second aunt were also described having contractures and joint stiffness, showing a clear autosomal dominant pattern of inheritance.
On clinical examination the proband showed palpebral ptosis, vertical ophthalmoplegia, mild weakness of some muscle groups (wrist extension and flexion, ankle flexion, neck flexion, shoulder abduction), spinal rigidity and joint contractures with limited movements (no wrist extension, limited finger extension and flexion, restriction of elbow flexion).
All affected family members showed, in addition to finger contractures with absent flexion of the distal phalanges, ophthalmoplegia and reduced respiratory capacity (FVC between 29% and 50%) without any

respiratory symptoms. Two of them needed spinal surgery because of a severe scoliosis. CK in the proband was within normal limits. An EMG and a muscle biopsy in his father did not show any specific abnormalities. Genetic testing for *LMNA*, *MHY7* and *MHY2* was negative. The family was included in a whole exome sequencing project, which revealed a heterozygous missense mutation in *PIEZO2* that was confirmed in all affected family members by Sanger sequencing.
The *PIEZO2* gene codes for a mechanically activated ion channel recently associated to two severe conditions, Marden-Walker syndrome and Gordon syndrome, and to a subtype of distal arthrogryposis (Coste B. et al 2013, McMillin M. et al 2014).
Here we report a family with a novel mutation in the *PIEZO2* gene and an extended distal arthrogryposis type 5A phenotype with ophthalmoplegia and restrictive respiratory function.

P122

Modelling a novel *INPP5K* mutation associated with Marinesco-Sjögren syndrome in zebrafish

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Background: Marinesco-Sjögren syndrome (MSS) is a rare autosomal recessive disorder characterised by congenital cataracts, progressive myopathy, cerebellar ataxia, mental retardation, and short stature. A cohort of MSS patients with a clinical phenotype of MSS but without mutations in SIL1, were analysed by whole exome sequencing. A potentially novel mutation in the protein Inositol polyphosphate-5-phosphatase K gene (*INPP5K*) was identified. *INPP5K* is believed to localise in the cytosol and is abundant in skeletal muscle, heart, and the kidneys. In order to demonstrate the role of *INPP5K* mutations as a novel cause of MSS, we are performing *in vivo* functional studies using zebrafish.
Methods: Two orthologues of *INPP5K* exist in zebrafish, on chromosome 10 (*INPP5K1*) and chromosome 15 (*INPP5K2*). Two different splice donor site morpholino antisense oligonucleotides (MO) were designed to target exon 4 of both *INPP5K1* & *INPP5K* to knockdown *INPP5K* gene expression in the zebrafish Golden strain ($slc24a5^{b1/+}$). Cryosectioning with toluidine blue staining of eyes and antibody staining of muscle and the neuromuscular junction (NMJ) were used to visualise defects in knockdown fish.
Results and Conclusions: The pathology observed in *INPP5K* knockdown fish can be attributed to malformations in muscle and eye structure during early development. *INPP5K* injected embryos with compromised motility have normal NMJ structure providing evidence that impaired motility is due to musculature abnormalities. The MSS patients exhibit a clinical phenotype consisting of congenital cataracts and myopathy. The evidence presented supports the hypothesis that a mutation in the human *INPP5K* could be the causative mutation in some patients with MSS.

P123

OPTIMISTIC: Observational Prolonged Trial In Myotonic dystrophy type 1 to Improve Quality of Life-Standards, a target identification collaboration

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[1]Newcastle University, UK; [2]Collaboration of 9 partners across Europe (L'Assistance Publique – Hopitaux de Paris, Radboud University Medical Centre, University of Glasgow, Ludwig-Maximilians University Munich, University of Aberdeen, Tayside Clinical Trials Unit, University of Dundee, Concentris gmbh)

Background: Myotonic Dystrophy Type 1 (DM1) is the most common form of muscular dystrophy in adults with an estimated prevalence of approximately 10 per 100,000. Fatigue is documented as the most common non muscular complaint of the condition, impacting on physical and social functioning as well as on mental and general health. Lack of physical activity and reduced initiative contribute to the experienced fatigue, which is a major determinant of quality of life.

Aims: OPTIMISTIC is an EU funded clinical trial testing a unique cognitive behavioural therapy with the aim of increasing activity, reducing fatigue and improving quality of life in people with DM1. Other key objectives include providing outcome measures that are relevant for the patients and have a rate of change that is appropriate for a clinical trial timeframe. OPTIMISTIC will also identify genetic factors that predict outcome and potential biomarkers as surrogate outcome measures that best explain the observed clinical variation.
Methods: A total of 286 patients will be recruited into this EU-funded assessor blind, randomised trial. The intervention is a unique combination of CBT and exercise, focussing on increasing the participants activity. Along each patients participation period of 17 months, five assessments visits will be performed to evaluate outcomes of Fatigue, Activity, Quality of Life, Mood and Cognitive capacity.
Results and Conclusion: An on-going trial that has marked an important step forward in the field of intervention for myotonic dystrophy type 1. OPTIMISTIC is engaged to address and collaborate with the wider neuromuscular community including patient organisations and join efforts to find new therapies for this disease by 2020.

P124

Identification of causal genes in congenital myasthenic syndrome by whole exome sequencing

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Background: Congenital myasthenic syndromes (CMS) are a group of genetically heterogeneous disorders characterised by compromised function at the NMJ. CMS manifests in childhood with fatigable weakness of limb, ocular and bulbar muscles. There are 20% patients with a clinical diagnosis that still remain genetically undiagnosed despite over 20 known CMS genes.
Aims: To identify novel CMS genes by whole exome sequencing (WES).
Methods: DNA from a proband with clinical diagnosis of CMS was sent to deCODE genetics for WES with suspected autosomal recessive inheritance. Variants in the exome were filtered to exclude those with a frequency greater than 1%, unlikely to significantly impact the protein and not compatible with the inheritance model. Genes that had not been excluded were then segregated in the family. Following segregation there was one remaining candidate (SLC12A8), which was then analysed in zebrafish. The zebrafish orthologue identified (slc12a8) was knocked down via antisense morpholino oligonucleotide (MO) injection and phenotype investigated.
Results: SLC12A8 was the only gene to segregate in this family. MO injection caused retention of intron 3 of slc12a8, leading to lack of movement in response to tactile stimulation, shortening and abnormal branching of motor axons, poorly defined somites and disorganisation of muscle fibres. No effect on sensory nerves or the CNS was observed.
Conclusion: SLC12A8 has been identified as a possible causative gene in CMS by WES. The preliminary studies carried out on zebrafish with slc12a8 knockdown demonstrated phenotypes consistent with CMS such as disorganisation of muscle architecture and abnormal branching of motor axons. To confirm results obtained using MO injection, we are now utilising CRISPR technology to target the gene in zebrafish.

Current UK Neuromuscular Clinical Trials

MRC Centre CTIMPs Set-up Phase trials

1. A Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Infantile-onset Spinal Muscular Atrophy
Sponsor: ISIS PHARMACEUTICALS, INC.
Planned start date: April 2015
London
PI: Prof. Francesco Muntoni
Recruitment target: 2-3
Newcastle:
PI: Prof. Volker Straub
Recruitment target: 1
This randomized, double-blind, sham-procedure controlled study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of intrathecal ISIS 396443 over 13 months. Approximately 111 subjects will be randomized in a 2:1 ratio (74 ISIS 396443: 37 control) to receive ISIS 396443 by intrathecal lumbar puncture (LP) injection or to a sham-procedure control. A scaled equivalent dose of 12 mg ISIS 396443 will be given at each of 6 times (i.e., on Study Days 1, 15, 29, 64, 183, and 302). The primary endpoint for this study is time to death or permanent ventilation [≥16 hours ventilation/day continuously for >21 days in the absence of an acute reversible event OR tracheostomy]

2. Phase 3, Randomized, Double-blind, Sham-Procedure Controlled Study to Assess the Clinical Efficacy and Safety of ISIS 396443 Administered Intrathecally in Patients with Later-onset Spinal Muscular Atrophy
Sponsor: ISIS PHARMACEUTICALS, INC
PI: Prof. Volker Straub
Recruitment target: to be determined
This randomized, double-blind, sham-procedure controlled study will test the clinical efficacy, safety, tolerability, and pharmacokinetics of intrathecal ISIS 396443 over 15 months. Approximately 117 subjects will be randomized in a 2:1 ratio (78 ISIS 396443: 39 control) to receive ISIS 396443 by intrathecal lumbar puncture (LP) injection or to a sham-procedure control. A dose of 12 mg ISIS 396443 will be given at each of 4 times over the 15 months (i.e., on Study Days 1, 29, 85, and 274).

3. Phase 1b/2, double-blind, placebo-controlled, within-subject, dose escalation study to evaluate the safety, efficacy, pharmacokinetics and pharmacodynamics of PF-06252616 administered to ambulatory boys with Duchenne Muscular Dystrophy.
Sponsor: PFIZER
Planned start date: May 2015
Recruitment target: 3-5
Newcastle:
PI: Prof. Volker Straub
London
PI: Prof. Francesco Muntoni
Recruitment target: 3
This is a Phase 2 randomized, 2-period, double-blind, placebo-controlled, multiple ascending dose study to evaluate the safety, efficacy, PK and PD of PF-06252616 administered to ambulatory boys diagnosed with DMD. Three IV infused dose levels (5, 20, 40 mg/kg) administered every 28 days will be investigated in a within subject dose escalating fashion. Approximately 105 eligible subjects will be randomly assigned to 1 of 3 sequence groups for approximately 96 weeks (2 treatment periods of approximately 48 weeks each) stratified by their baseline time to complete the 4 stair climb.

4. A Phase 3 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of Sialic Acid Extended Release Tablets in Patients with GNE Myopathy (GNEM) or Hereditary Inclusion Body Myopathy (HIBM)
Sponsor: Ultragenyx
Newcastle:
PI: Prof. Hanns Lochmuller
Recruitment target: 15-20
This is a randomized, double-blind, placebo-controlled, multicenter study to assess the clinical effect of 6 g/day SA-ER treatment as compared with placebo in subjects with GNEM. Approximately 80 subjects will be randomized in a 1:1 ratio to receive 6 g/day of SA-ER or matching placebo for 48 weeks. Randomization of subjects will be stratified by gender with a planned enrolment of no more than 60% of subjects of either gender.

5. A double-blinded, randomised controlled trial to determine the effects of high versus low dose testosterone treatment of pubertal delay in Duchenne Muscular Dystrophy
Sponsor: Newcastle upon Tyne NHS Hospitals Foundation Trust
PI: Prof. Volker Straub
Recruitment target: 24
This is a non-commercial, phase 3 study to determine the acceptability and clinical effectiveness of high dose versus low dose testosterone treatment of pubertal delay in boys with Duchenne Muscular Dystrophy. In order to satisfactorily address the primary objective, a mixed methods research approach will be required, combining methods from both qualitative and quantitative paradigms. According to Greene et al, there are four major advantages of a mixed methods approach: enhanced validity and credibility; greater comprehensiveness of findings; more insightful understandings and increased value consciousness and diversity. The quantitative data obtained from the TSQM will be used to guide the qualitative data collection in a 'sequential explanatory design'. The qualitative component of the study will comprise semi-structured interviews that will be carried out on all subjects at the end of the study period.

6. A multicenter, randomized, double-blind, placebo controlled efficacy and safety trial of intravenous zoledronic acid twice yearly compared to placebo in osteoporotic children treated with glucocorticoids
Sponsor: Novartis
PI: Dr Ros Quinlivan
Recruitment target: 3
Evaluate the efficacy and safety of zoledronic acid plus vitamin D and calcium compared to placebo plus vitamin D and calcium in osteoporotic children treated with glucocorticoids. The primary efficacy objective of the study is to demonstrate that zoledronic acid administered every 6 months 0.05 mg/kg (max 5 mg) is superior to placebo for the change in lumbar spine (LS) areal bone mineral density (BMD) Z-score at Month 12 relative to baseline.

7. 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
Recruitment target: 12
Andersen-Tawil Syndrome (ATS) is a rare form of periodic paralysis that is associated with serious heart-rhythm abnormalities. ATS is characterized by a triad of episodic muscle weakness, long-QT syndrome with potentially fatal cardiac dysrhythmias and skeletal developmental anomalies. The underlying cause of this potentially fatal condition is only partly understood and there are no established treatments. Mutations in the KCNJ2 gene encoding Kir2.1, an inward-rectifying potassium channel account for approximately 60% of ATS cases (termed ATS1), the remaining 40% are presumed to have an as yet undetermined gene lesion and are designated ATS2. ATS1 and ATS2 are phenotypically indistinguishable. The treatment of ATS has been largely anecdotal and empirical.

This proposal involves a multi-centre, placebo-controlled 'n of 1' study design of total duration 45 weeks. The expected total enrolment for this multi-centre study is 16 participants. The aim of this study is to determine whether potassium supplements and/or acetazolamide alter the duration of muscle weakness and potentially life-threatening heart rhythm abnormalities in patients with ATS.

8. Eplerenone versus triamterene in CAI non-responsive periodic paralysis (HOP Study)
Status: Set-up phase
Sponsor: UCL
Funder: MDA
Planned start date: TBC
PI: Prof. Michael Hanna
Recruitment target: 11
There have been no previous systematic investigations of patients with hypokalemic periodic paralysis who fail to respond or are worsened by carbonic anhydrase inhibitors. The initial impression that only 10-15% of patients fall into this category now appears erroneous (Matthews, Portaro et al. 2011, Neurology). Approximately 50% of genetically-confirmed patients with periodic paralysis do not derive sufficient benefit to remain on treatment. By pursuing a pilot study of alternative treatments for this subgroup of patients we hope to start addressing what is arguably the greatest need of the community of patients with the periodic paralyses.

Specific Aims

1) To obtain preliminary efficacy data of triamterene and eplerenone in HOP patients unresponsive to or unable to tolerate carbonic anhydrase inhibitors. To achieve these aims, we will perform an 18 week, 2 centres, randomized, double-blind, placebo controlled crossover pilot trial with eplerenone and triamterene versus placebo in CAI non-responsive HOP patients. Patients with clinically well documented HOP who have defined mutations in the Na or Ca channels and who have worsened with, not responded to or not been able to tolerate CAI, will be studied. Following a 4 weeks run in period (phase 1) 22 patients will receive 4 weeks placebo, 4 weeks eplerenone and 4 weeks triamterene in a randomized fashion. Each 4 weeks period will be separated by a 1 week washout period. Improvement in attack rate, severity weighted attack rate, and quality of life will be measured.
2) To select the drug, triamterene or eplerenone, with the optimal efficacy and adverse effect profile for a future larger trial.

9. A phase IIb/III of Arimoclomol in IBM
Status: Set-up phase
Sponsor: UCL
Funder: FDA/Orphazyme (TBC)
Planned start date: TBC
PI: Prof. Michael Hanna
Recruitment target: 150
(This is a follow-up of the phase IIa RCT study concluded in 2012)
We are proposing a one year randomized, placebo-controlled Phase IIb/III study of arimoclomol in 150 IBM subject. The primary aim is to assess the efficacy and safety of arimoclomol (200 mg TID). The primary efficacy endpoint is the IBMFRS. Secondary efficacy outcomes will include different measures of strength and function: manual muscle testing (MMT), maximum voluntary isometric contraction (MVICT), timed up and go (TUG), timed 10 meter walk test, 6 minute walk test, Purdue pegboard test, grip and pinch test; a general physical function measure: Health

Assessment Questionnaire (HAQ- DI); a HRQoL measure using SF36 and MRI acute thigh pathology (oedema), chronic pathology (fat fraction) and muscle volume. Safety laboratory and adverse events will be collected. Our long-term goal is to find an effective treatment for people with IBM.
For further information please contact Dr Pedro Machado at p.machado@ucl.ac.uk

10. A double-blind, randomised, multicenter, placebo-controlled, parallel-group study to evaluate the efficacy and safety of fingolimod 0.5 mg administered orally once daily versus placebo in patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)
Status: Recruitment to start March 2015
Sponsor: Novartis
PI: Dr Michael Lunn
Recruitment target: 1-2
The study is designed to evaluate the efficacy and safety of fingolimod in the treatment of CIDP compared with placebo. Data from this study will be used to support the registration of fingolimod in the indication of CIDP.
This is a double-blind, randomised, multicenter, placebo-controlled, parallel-group study in patients with a confirmed diagnosis of CIDP and treated IVIg, corticosteroids, or both therapies prior to study entry. Patients meeting the eligibility criteria will be randomly assigned in a ration of 1:1 to receive oral fingolimod (0.5 mg/day) or matching placebo.

The study will consist of 3 periods: a Screening Period (lasting for up to 45 days), a Double-blind Treatment Period (variable duration on pre-specified rules, but not exceeding 3 years after study initiation), and a Follow-up Period after discontinuation of study drug treatment. Patients who complete the study will have an option to enter an extension study.

The duration of this study is flexible, based on pre-specified rules. Under the current assumption the trial duration is anticipated to be two years from the study initiation but should not exceed approximately three years.

11. Stratifying Patients with Leber Hereditary Optic Neuropathy (LHON) for Idebenone Therapy Trial (SPLI-TT)
Status: set to recruit March 2015
Funder: EME-MRC
PI: Prof. Patrick Chinnery
The study objective is to carry out a double-blind patient-randomised placebo-controlled trial in LHON patients stratified by their genetic status (i.e. the presence of the m.11778G>A, m.14484T>C or m.3460G>A mtDNA mutations). This is in order to discover if mtDNA analysis can be used to identify patients with LHON whose vision is most likely to improve with Raxone® treatment, and if Raxone® stabilise or improve vision in a genetically-defined group of patients with LHON harbouring the m.11778G>A, m.14484T>C or m.3460G>A mtDNA mutations.

12. NIHR Pain Consortium (Bridge Neuropathic Pain)
Status: Set up. Due to start later 2015
Funder: NIHR BioResource – Rare Diseases
Sponsor: Cambridge University Hospitals NHS Foundation Trust & University of Cambridge
PI: Prof. Mary M. Reilly
The overall aim of the project is to perform whole genome sequencing on 1000 patients with a neuropathic pain phenotype.

It is increasingly recognised that high impact gene variants are responsible for heritable disorders of pain sensing both gain (e.g. Inherited erythromelalgia) and loss (congenital insensitivity to pain) of function. Furthermore conditions which were recently thought to be

acquired are now known to have a genetic basis in a significant proportion of cases (small fibre neuropathy).
This application not only has relevance for diagnosis but also for tailoring treatment appropriately for these disabling conditions. This application has catalysed the formation of clinical pain research collaboration of the Biomedical Research Centres of Cambridge, Guys/St Thomas /Kings College, Great Ormond Street, Imperial, Oxford, Royal Marsden and UCL. This is a multidisciplinary grouping of clinician scientists dealing with chronic pain including pain physicians, neurologists, dentists, paediatricians and clinical geneticists, and will harness existing clinical phenotype facilities of the respective BRCs.
This will enable critical mass in collecting clinical pain cohorts of significant size, facilitate multidisciplinary expertise in both genetic analysis and clinical phenotyping and allow us to study the role of high impact gene variants across distinct pain disorders.
The collaboration will:

1. Aim to find the genetic basis for inherited pain disorders for which causative locus is unknown.
2. Aim to reduce the delay in making genetic pain diagnoses, e.g. small fibre neuropathy.
3. Aim to discover genotype/phenotype correlations enabling patient stratification to predict prognosis and treatment response.

MRC Centre CTIMPs Open Trials

13. GSK/Prosensa clinical trial in DMD boys with study drug GSK2402968 (GSK Extension Study)
Full Title: An open-label extension study of the long-term safety, tolerability and efficacy of GSK2402968 in subjects with Duchenne Muscular Dystrophy.
Status: Closed to recruitment/ Dosing suspended
Sponsor: GlaxoSmithKline
Funder: GlaxoSmithKline
Newcastle
CI/PI: Prof. Volker Straub
London
PI: Prof. Francesco Muntoni
Patients target: 8; recruited: 8
Description: A Phase III, multicenter, open-label extension, study in male outpatients with Duchenne Muscular Dystrophy (DMD) who have participated in either DMD114117 or DMD114044. All subjects will receive 6mg/kg GSK2402968 weekly for a minimum period of two years or an intermittent dosing frequency of 6mg/kgGSK2402968 for a minimum period of two years.
Objective(s)
Primary objective:
• To evaluate the long term safety, tolerability and efficacy of subcutaneous 6mg/kg/weekGSK2402968 in subjects with DMD who have participated in either DMD114117 orDMD114044.
Secondary objectives:
• To evaluate the long-term PK of subcutaneous 6 mg/kg/week GSK2402968 in subjects with DMD who have participated in either DMD114117 or DMD114044.
• To evaluate the long-term impact on health-related quality of life (HRQoL) and functional outcomes of continued treatment with GSK2402968 in subjects with DMD who have participated in either DMD114117 or DMD114044.
• To evaluate DMD disease progression and outcomes (clinical, HRQoL and functional) in subjects who discontinue active treatment during the conduct of study (natural history component).
• To evaluate the long-term safety, efficacy and PK of an intermittent dosing option in those subjects unable to tolerate GSK2402968 6mg/kg/week dosing.

This study aims to enrol approximately 200 subjects. In the primary dosing arm, subjects will receive GSK2402968 6 mg/kg as subcutaneous injections once a week for a period of 104 weeks. Further information about this study can be obtained from the MRC Centre Clinical Trials Coordinator on 020 7905 2639.

14. 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: Closed to recruitment
Sponsor: Newcastle upon Tyne NHS Hospitals Foundation Trust
Funder: British Heart Foundation
Newcastle
PI: Dr John Burke
Recruitment target: 20-30; recruited: 26
London
PI: Prof. Francesco Muntoni
Recruitment target: 50-60; recruited: 46
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 ACE inhibitor and beta-blocker therapy (perindopril and bisoprolol) over a minimum of three years and a maximum of five years. 140 participants (70 per arm) are to be enrolled and randomised. For more information about the study please contact the trial coordinator on 020 7905 2639.

15. FOR-DMD
Full Title: Duchenne muscular dystrophy: double-blind randomized trial to find optimum steroid regimen (FOR-DMD)
Status: Open to recruitment
Sponsor: University of Rochester
Funder: NIH
Newcastle
CI: Prof. Kate Bushby
PI: Prof. Volker Straub

Recruitment target: 8; recruited: 7
London
PI: Prof. Francesco Muntoni,
Recruitment target: 8; recruited: 5
This is a multi-centre, double-blind, parallel group, 36-60 month study, comparing three corticosteroid regimens in wide use in DMD:

- daily prednisone (0.75 mg/kg/day)
- intermittent prednisone (0.75 mg/kg/day, 10 days on, 10 days off)
- daily deflazacort (0.9 mg/kg/day)

Primary study objective: The proposed randomized controlled trial will compare 3 corticosteroid regimens to address the pragmatic hypothesis that daily corticosteroids (prednisone or deflazacort) will be of greater benefit in terms of function and subject/parent satisfaction than intermittent corticosteroids (prednisone).
Secondary study objectives: A second hypothesis is that daily deflazacort will be associated with a better side effect profile than daily prednisone. The study protocol includes standardized regimens for prevention/ treatment of predictable side effects of corticosteroid medication, as well as standards of care for the general management of DMD. The trial directly addresses the current chaos in prescribed treatment schedules; its results will have direct impact on the current and future management of boys with DMD throughout the world by providing the evidence base for rational clinical practice.
The results of the trial will allow the generation of clear and specific evidence-based guidelines for patient treatment.

16. PTC124-GD-019 Open label
Full-Title: An open-label study for previously treated Ataluren (PTC124®) patients with nonsense mutation dystrophinopathy
Status: **Closed to recruitment**
Sponsor& Funder: PTC
Newcastle
CI/PI: Prof. Kate Bushby
London
PI: Prof. Francesco Muntoni
Patients recruited: 8 (GOSH), 11 (Newcastle)
This study comprises a phase III, open-label study of ataluren in patients with nmDBMD who previously received ataluren at an investigator site in a prior PTC-sponsored clinical study. Subjects will receive ataluren 3 times per day (TID) at respective morning, midday, and evening doses of 10 mg/kg, 10 mg/kg, and 20 mg/kg, for approximately 96 weeks. Study assessments will be performed at clinic visits during screening, on the first day of ataluren dosing, and then every 12 weeks during the ataluren treatment period.
Primary Objective:
The primary objective of this study is to assess the long-term safety and tolerability of 10, 10, 20 mg/kg ataluren in patients with nmDBMD who had prior exposure to ataluren in a PTC-sponsored clinical trial.
Secondary objectives include the following:

- Ambulatory patients (able to run/walk 10 meters in ≤30 seconds) - To determine the effect of

ataluren on ambulation and other aspects of physical function

- Nonambulatory patients (unable to run/walk 10 meters in ≤30 seconds) - To assess the effect

of ataluren on activities of daily living, upper limb function, and pulmonary function

- All patients – To assess patient and/or parent/caregiver reports of changes in disease status:

Retrospectively during and after participation in previous studies (Studies 007 and 007e) and prospectively during the current study.

17. Full Title: A phase III efficacy & safety study of Ataluren (PTC124) in patients with nonsense mutation dystrophinopathy (PTC Phase III) PTC124-GD-020-DMD
Status: Closed to recruitment
Sponsor: PTC
Funder: PTC
Newcastle
CI/PI: Prof. Kate Bushby
London
PI: Prof. Francesco Muntoni,
Patients recruited: 7 (GOSH) 4 (Newcastle)

A phase 3 efficacy and safety study of ataluren (ptc124) in patients with nonsense mutation dystrophinopathy

The primary objective of this study is to determine the ability of ataluren to slow disease progression as assessed by ambulatory decline (decrease in 6MWD) in patients with nonsense mutation dystrophinopathy.
Secondary endpoints have been chosen to evaluate changes in skeletal muscle function through assessment of proximal muscle function, as assessed by the time to run/walk 10 meters, time to ascend 4 stairs and time to descend 4 stairs and patient or parent/caregiver perception of physical functioning. Additional secondary endpoints have been selected to enhance understanding of the primary and secondary treatment effects. For example, a beneficial effect in physical function relative to placebo, as assessed by the North Star Ambulatory Assessment (NSAA), would compliment positive changes in ambulation proximal muscle function. Collection of patient and/or parent reported changes in disease status provides an opportunity to expand the implications of a drug effect on the patient's disease symptoms and activities of daily living.

18. Full Title: A phase IIb, open-label study to assess the efficacy, safety, pharmacodynamics and pharmacokinetics of multiple doses of PRO045 in subjects with Duchenne muscular dystrophy (PRO045)
Status: Open to recruitment
Sponsor: Prosensa
Funder: Prosensa
Newcastle
CI/PI: Prof. Volker Straub
London
PI: Prof. Francesco Muntoni,
Patients recruited: 1 (GOSH) 2 (Newcastle)
Primary objective: To assess the efficacy of PRO045 after 48 weeks treatment in ambulant subjects with Duchenne muscular dystrophy.
Secondary objectives: To assess the safety and tolerability of PRO045 after 48 weeks of treatment in all study subjects with Duchenne muscular dystrophy including subjects from the dose-escalation phase of the study. To determine the pharmacokinetics of PRO045 at different dose levels after subcutaneous administration in subjects with Duchenne muscular dystrophy.
To assess the pharmacokinetics, bioavailability and safety of PRO045 following single intravenous dose administration at different dose levels.
To assess the pharmacodynamics of PRO045 at different dose levels after subcutaneous administration in subjects with Duchenne muscular dystrophy.
To assess trend in efficacy in all subjects with Duchenne Muscular Dystrophy not included in the primary objective after 48 weeks of treatment.

19. An Open-label, multicenter, multinational, ascending dose study of the safety, tolerability, pharmacokinetics, pharmacodynamics, and exploratory efficacy of

repeated biweekly infusions of neoGAA in naïve and alglucosidasealfa treated late-onset Pompe disease patients.
Status: Open to recruitment
Sponsor: Genzyme
Funder: Genzyme
PI: Prof. Volker Straub
Patient recruited: 1
Phase I, multicenter, multinational, open-label, ascending dose, repeated bi-weekly intravenous infusion study of neoGAA in:
• Group 1 – Late-onset Pompe disease patients naïve to treatment, 3 dose levels
• Group 2 – Late-onset Pompe disease patients previously treated with alglucosidasealfa, 3 dose levels

Objectives:
Group 1
To determine in treatment naïve patients with late-onset Pompe disease patients:
• The safety and tolerability of neoGAA
• The pharmacokinetic parameters of neoGAA
• The pharmacodynamic effects of neoGAA on skeletal muscle and other exploratory biomarkers
• The effect of neoGAA on exploratory efficacy endpoints
Group 2
To determine in alglucosidasealfa treated late-onset Pompe disease patients:
• The safety and tolerability of neoGAA
• The pharmacokinetic parameters of neoGAA
• The pharmacodynamic effects of neoGAA on skeletal muscle and other exploratory biomarkers
• The effect of neoGAA on exploratory efficacy endpoints

20. A randomized, double-blind, placebo-controlled, multicenter, parallel group, dose-finding, pivotal, phase IIb/III study to evaluate the efficacy, safety and tolerability of intravenous BYM338 at 52 weeks on physical function, muscle strength, and mobility and additional long-term safety up to 2 years in patients with sporadic inclusion body myositis
Sponsor: Novartis
Status: Closed to recruitment
CI: Prof. Michael Hanna
PIs: Hector Chinoy; James Miller
Patients recruited: 44 (UK); 353 (WW)
The purpose of this dose-finding study is to demonstrate that at least one dose regimen of BYM338 in sporadic inclusion body myositis (sIBM) patients improves physical function and mobility when compared to placebo after 52 weeks of treatment. The study will assess efficacy, safety, tolerability and pharmacodynamic effect of i.v. administration of BYM338 compared to placebo on lean body mass, muscle strength, physical function and mobility in sIBM patients. The results will support marketing authorization applications for BYM338 as treatment for sIBM patients.
This is a multi-center, pivotal, randomized, double-blind, placebo-controlled, 4 arm dose-finding, phase IIb/III trial.
For more information contact Dr Pedro Machado: p.machado@ucl.ac.uk

21. A Phase 2/3 Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of ISIS 420915 in Patients with Familial Amyloid Polyneuropathy
Sponsor: ISIS Pharmaceutical
Status: Recruiting
PI: Prof. Mary M. Reilly
Global Recruitment target: 195

Multicentre, randomized, double-blind, placebo-controlled study. Approximately 195 patients will be randomized in a 2:1 ratio (130 ISIS 420915 and 65 PBO) to receive 300 mg ISIS 420915 or placebo. Study Drug (ISIS 420915 or placebo) will be administered three times on alternate days during Week 1 (Days 1, 3 and 5), and then once weekly during Weeks 2-65 (for a total of 67 doses). Patients will also receive daily supplemental doses of the recommended daily allowance of vitamin A. The end of treatment (EOT) efficacy assessment is conducted at Week 66. Following treatment and the EOT efficacy assessment, eligible patients (including patients that received placebo), may elect to enrol in an open-label extension (OLE) study pending study approval by the IRB/IEC and the appropriate regulatory authority. All participating patients in the OLE study will receive 300 mg ISIS 420915 once weekly. Otherwise, patients will enter the 6 month post-treatment evaluation portion of the study.

22. A Pilot Study of Valproate Sodium for McArdle Disease
Status: Open to recruitment
Sponsor: UCL
Funder: Muscular Dystrophy campaign
PI: Dr Ros Quinlivan
Recruitment target: 15
McArdle disease (Glycogen storage disease type V, GSDV) is an inherited metabolic disorder of skeletal muscle. Affected patients are unable to produce lactate during ischaemic exercise [McArdle 1951] because they have a congenital absence of the enzyme muscle glycogen phosphorylase, which is essential for glycogen metabolism [Mommaerts 1959, Schmidt and Mahler 1959]. The condition is caused by homozygous or compound heterozygous mutations in the muscle glycogen phosphorylase gene *(PYGM)* located at chromosome 11q13 [Beynon 2002]. This enzyme deficiency results in the inability to mobilise muscle glycogen stores that are normally required for energy during anaerobic metabolism. In affected people, symptoms of fatigue and cramp occur within minutes of initiating any activity and during strenuous activity such as lifting heavy weights or walking uphill, if the activity is continued despite severe cramping, a contracture occurs which leads to muscle damage (rhabdomyolysis), myoglobinuria and, when severe, acute renal failure.

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

Although most people with McArdle disease have complete absence of skeletal muscle phosphorylase, there are a small minority of patients who possess splice site mutations that enable production of very small amounts (1-2%) of functional enzyme [Vissing]. These people have a milder phenotype with less severe symptoms, and functional exercise assessments have shown better exercise capacity than typical patients with the condition. Findings from these atypical individuals suggest potential therapeutic agents might only need to produce very small amounts of enzyme for significant functional improvement. Furthermore, finding a therapeutic agent to 'switch on' expression of the foetal isoenzyme may be a potential therapeutic strategy. Sodium Valproate (Valproic acid) is one of a group of drugs known as histone deacetylase inhibitors (HDACIs) that can affect gene expression by acetylating lysine residues, which in turn has a direct effect on chromatin [Thiagalingam 2003]. There is some evidence from animal studies to suggest that sodium valproate can 'switch on' the foetal phosphorylase isoenzyme. A recent clinical trial of the drug in McArdle sheep that were given sodium valproate for three months showed the presence of phosphorylase positive muscle fibres, in the absence of muscle necrosis and/or regeneration [Howell 2010].
The current proposes an open label uncontrolled pilot study to evaluate safety and efficacy of Sodium valproate (slow release) 20mg /kg once daily for six months. 15 subjects, adult male and post menopausal women attending specialist centres for McArdle disease will be recruited across three sites: London, Copenhagen and Dallas.

23. Bumetanide in HypoPP
A randomised, double-blind, placebo-controlled, phase II clinical trial with a cross-over design assessing efficacy of a single dose of bumetanide in reducing focal attack severity in hypokalaemic periodic paralysis assessed using the McManis protocol
Status: Open to recruitment
Sponsor: UCL
Funder: UCL Charities
PI: Dr Doreen Fialho
Recruitment target: 12
This is a planned phase II clinical trial, double-blind, randomised, placebo-controlled cross-over, single-site study to investigate the efficacy of bumetanide in patients with hypokalemic periodic paralysis (HypoPP). The objective is to assess the efficacy of bumetanide in reducing severity and duration of acute attacks of weakness in HypoPP patients. Hypokalaemic periodic paralysis is an autosomal dominant muscle channelopathy with onset in the first or second decade, characterized by attacks of reversible flaccid paralysis lasting from several hours to days. These patients may have frequent attacks of weakness interfering with daily activities and work, and are often hospitalized for intravenous potassium treatment causing a significant economic burden. They may also progress to a chronic myopathy especially because there are no optimal treatments available nowadays.
Experimental evidence of the use of bumetanide in a mouse model of HypoPP has provided convincing evidence that it can abort paralytic attacks.
We would expect bumetanide to abort acute attacks of weakness faster and reduce their severity, reducing the likelihood of patients being hospitalized during severe attacks. Bumetanide will add as an adjuvant therapy to potassium intake during an attack.

24. Multicenter, open-label extension study to investigate the long-term safety and efficacy of IgPro20 in maintenance treatment of chronic inflammatory demyelinating polyneuropathy (CIDP) in subjects completing study IgPro20_3003 (PATH extension Study)
Status: Open to recruitment
Sponsor: CSL Behring
PI: Dr Michael Lunn
Patient target: 3
The current study is an extension study to the pivotal study IgPro20_3003. Clinical studies have demonstrated the clinical efficacy and safety of using IVIGs to treat CIDP (Kieser et al., 2008; Eftimov et al., 2009: Hughes at al., 2008). Study IgPr20_3003 is being conducted to provide evidence of subcutaneous immunoglobulin (SCIG) as an alternative treatment option for CIDP in demonstrating safety and efficacy of IgPro20 as maintenance therapy in subjects treated with IVIG and switched to SCIG.

Methodology:

This is an open-label prospective, multicenter extension study for subjects who have participated in the subcutaneous (SC) Treatment Period of the preceding pivotal CIDP study IgPro20_3003. Subjects may either transition directly from study Igpro20-3003 to IgPr20-3004 or with an interval (1 - For subjects who had the completion visit in study IgPro20_3003 before IgPro20_3004 study was open for enrolment at the subject's site, enrolment is not later than 8 weeks after study IgPro203004 is open for enrolment at the subject's site; 2- For subjects who completed study IgPro20_3003 after IgPro20_3004 study was open for enrolment at the subject's study site, enrolment is not later than 8 weeks after completion visit of study IgPro20_3003 for this subject).

Subjects who receive IgG between studies IgPro20_3003 and IgPro20_3004 should be enrolled within 1 week after the last administration of IgG.

25. Pro053: Title: A Phase I/II, open-label, dose escalating with 48-week treatment study to assess the safety and tolerability, pharmacokinetics, pharmacodynamics and efficacy of PRO053 in subjects with Duchenne muscular dystrophy
Status: Closed to Recruitment
Sponsor & Funder: Prosensa
Newcastle:
CI: Prof. Volker Straub
PI: Prof. Volker Straub
Target 3-5, recruited 1
London:
PI: Prof. Francesco Muntoni
Target 1-2; recruited: 1
The study population will be comprised of male subjects with Duchenne muscular dystrophy resulting from an exon deletion correctable by treatment with PRO053. A total of 42 male subjects are planned to be recruited, 6 of whom will participate in the dose-escalation and 48-week treatment phases, whilst the remaining 36 subjects will only participate in the 48-week treatment phase, including the dose selection part of the study.
Ambulant subjects who are able to walk at least 230 metres in 6 minutes will be included in the study as they are expected remain ambulant for at least a year (see Section 3.2).
The primary objective is to determine benefit of PRO053 treatment by assessing the 6MWD after 48 weeks on the selected dose in 30 planned evaluable subjects. To be evaluable, subjects must be able to walk at least 230 metres in 6 minutes at the start of the treatment phase once the optimum dose has been selected. Subjects from the dose-escalation phase and the dose selection phase will continue into the treatment phase for safety and secondary efficacy analysis only.
Subjects who withdraw from the treatment phase prior to 6 weeks of dosing due to non-safety or tolerability related reasons will be replaced.

26. A 2-Part, Randomized, Double-Blind, Placebo-Controlled, Dose-Titration, Safety, Tolerability, and Pharmacokinetics Study (Part 1) Followed by an Open-Label Efficacy and Safety Evaluation (Part 2) of SRP-4053 in Patients with Duchenne Muscular Dystrophy (DMD) Amenable to Exon 53 Skipping.
Sponsor: Sarepta, EU Grant
Status: Recruiting
London:
CI/PI: Prof. Francesco Muntoni
Target 12, recruited 3
Newcastle:
PI: Prof. Volker Straub
Target 12, recruited 2
This is a first-in-human, multi-center, multiple-dose study to assess the safety, tolerability, efficacy, and pharmacokinetics of once weekly IV infusions of SRP-4053 in patients with genotypically-confirmed DMD with a deletion amenable to exon 53 skipping (e.g., 42-52, 45–52, 47–52, 48–52, 49–52, 50–52; 52; 54-58). This study will be conducted in 2 parts.
Part 1 is a randomized, double-blind, placebo-controlled, dose-titration evaluation to assess the safety, tolerability, and pharmacokinetics of four dose levels of SRP-4053 in 12 patients with DMD over approximately 12 weeks.
Part 2 is a 48-week, open-label evaluation to assess the efficacy and safety of the selected dose level of SRP-4053 determined in Part 1 compared with an untreated concurrent control group. All 12 treated patients from Part 1 plus an additional 12 new patients in Part 2 must have a clinical diagnosis of DMD confirmed by the finding of a genomic deletion amenable to exon 53 skipping. Untreated control patients in Part 2 (up to 24) will be DMD patients with genotypically-confirmed DMD who are not amenable to treatment by exon 53 skipping but otherwise meet the same eligibility criteria as treated patients new to Part 2.

Part 1 will be approximately 12 weeks in duration while Part 2 will last approximately 52 weeks. Including a 4 to 6-week Screening period, the total study duration (for patients participating in both Parts 1 and 2) will be approximately 70 weeks.

27. A Multi-Center, Randomized, Double-Blind, Placebo-Controlled, Multiple- Dose Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of RO6885247 following 12 Weeks treatment in Adult and Pediatric Patients with Spinal Muscular Atrophy
Sponsor: F. Hoffmann-La Roche Ltd
Status: Recruiting
Newcastle:
CI/PI: Prof. Hanns Lochmuller
Target 7, recruited 3
London – not open yet
PI: Prof. Francesco Muntoni
This is a multicenter, randomized, double-blind, 12-week, placebo-controlled multiple dose Phase Ib study to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics of RO6885247 in adult and pediatric patients with SMA. Up to approximately 48 male and female SMA patients between the ages of 2−55 years (inclusive) will be enrolled into this study and randomized in a 2:1 ratio to receive either RO6885247 or placebo.

28. A Phase 3 Extension Study of Ataluren (PTC124) in Patients with Nonsense Mutation Dystrophinopathy (PTC20e –PTC 20 is being extended)
Sponsor PTC therapeutics
Status: Open
Newcastle:
CI/PI: Prof. Kate Bushby
Recruitment target: 4, recruited: 4
PI: Prof. Francesco Muntoni
Recruitment target: 6-9; 2 recruited to date
Primary Objective: To evaluate the long-term safety of ataluren in boys with nonsense mutation dystrophinopathy, as determined by adverse events and laboratory abnormalities. All previously enrolled in PTC20 will be eligible to enter the extension.

29. SMT C11003 - A placebo-controlled, multi-centre, randomized, double-blind, 3-period dose escalation study to evaluate the PK and safety of SMT C1100 in paediatric patients with Duchenne muscular dystrophy (DMD) who follow a balanced diet.
Sponsor: Summit Corporation plc
PI: Prof. Francesco Muntoni
Status: Recruiting
Recruitment target: 4
This will be a double-blind placebo control study where boys will be randomly allocated to the 3 treatment sequence groups. Each patient will receive both doses of SMT C1100 (1250 mg twice daily (BID) and 2500 mg BID) in a dose escalating fashion, with placebo in the other study period. The study period in which they receive placebo is dependent on the treatment sequence they are allocated to. The primary objective is to determine the single and multiple oral dose PK of SMT C1100 and its metabolites in patients with DMD who follow a balanced diet.

<u>MRC Centre CTIMPs Completed Trials</u>

30. RANDOMISED DOUBLE-BLIND PLACEBO CONTROLLED TRIAL OF LONG-TERM ASCORBIC ACID TREATMENT IN CHARCOT-MARIE-TOOTH DISEASE TYPE 1A Status: Completed.
Sponsor: University College London
Funder: Muscular Dystrophy Campaign (MDC)
PI: Prof. Mary M. Reilly
Patients recruited: 50 target 50

Charcot-Marie-Tooth disease 1A (CMT1A) is associated with a duplication of the peripheral myelin protein 22 (PMP22) gene. To date there is no pharmacological treatment for CMT1A patients. Treatments and therapy for CMT is restricted to symptomatic treatments such as physiotherapy and surgery for skeletal deformities.
Recently, treatment with ascorbic acid (AA) has been shown to be effective for transgenic mice over-expressing PMP22, a model of the human disease. Treated animals had much less severe neuropathy as compared to untreated controls as shown by clinical and histological findings. Some clinical parameters even improved during treatment.
This is a phase III prospective, multi-centre, 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 MRC Centre for Neuromuscular Diseases. Paper published in *Lancet Neurology 2010*.

31. THERAPEUTIC TRIAL OF MEXILETINE IN NON-DYSTROPHIC MYOTONIA
Full Title: A Phase II Randomised, Double-Blind, Placebo controlled, Cross-Over Study to Investigate the Efficacy of Mexiletine in Patients with Non-Dystrophic Myotonia
Status: Completed
Sponsor: University College London (UCL)
Funder: Food and Drug Administration (FDA – USA)
PI: Prof. Michael Hanna
Patients recruited: 14; target 15
The non-dystrophic myotonia (NDM) is a group of rare neuromuscular disorders that causes episodes of muscle stiffness (known as myotonias) and paralysis. Predominantly the muscles of the face, hands and legs are affected. In addition to these episodes a permanent and debilitating muscle weakness can develop. The optimal treatment for these disorders is unknown. Non-dystrophic myotonias are due to abnormalities of ion channels present in skeletal muscle membranes. There is experimental evidence that drugs like mexiletine which block the abnormal function of these ion channels allow the muscle to perform normally. The study aims to test the efficacy of mexiletine in the treatment of the non-dystrophic myotonias. This proposal involves a multi-centre, double-blind, placebo-controlled cross over trial of total duration nine weeks. Fifteen participants have been enrolled in the UK at the MRC Centre.

This work has been completed and outcome data published in *JAMA (Volume 308, No.13, pages 1357 - 1365, October 2012).*

32. A PHASE IIb EFFICACY AND SAFETY STUDY OF PTC124 IN SUBJECTS WITH NONSENSE MUTATION-MEDIATED DUCHENNE AND BECKER MUSCULAR DYSTROPHY
Status: Completed
Sponsor: PTC Therapeutics
Funder: PTC Therapeutics
Newcastle
CI: Prof. Bushby
London
PI: Prof. Muntoni,
Patients recruited: 11
Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder affecting young boys. The condition is disabling and life-threatening. A small subset of boys are classified as having Becker muscular dystrophy (BMD), a phenotypically milder form of the dystrophic muscle disease.
In approximately 10 to 15% of boys with DMD and BMD the causative defect is the presence of a nonsense mutation in the dystrophin gene that truncates dystrophin protein production by introducing a premature stop codon into the dystrophin messenger ribonucleic acid (mRNA). PTC124 is a novel, orally bioavailable, small-molecule drug that promotes ribosomal read-through of mRNA containing a premature stop codon. Through this mechanism of action, PTC124

has the potential to overcome the genetic defect in boys for whom a nonsense mutation causes DMD/BMD.
In vitro studies in cell lines with dystrophin nonsense mutations have shown that PTC124 can restore production of the missing dystrophin gene.
This is an international, multi-centre, randomised, double-blind, placebo-controlled, dose-ranging, efficacy and safety study.
The study primary aim is to evaluate the effect of PTC124 on ambulation as assessed by the distance walked during a 6-minute walk test (6MWT).
The double-blind arm of the study randomised 174 participants worldwide which are to be followed for a period of 12 months. At the completion of the blinded treatment, all compliant participants were eligible to receive open-label PTC124 in a separate extension study.
(Ataluren is now the non-proprietary generic name for PTC124).
This work has been completed.
The preliminary findings from the Ataluren Study 007 did not show significant muscle improvement in the patients who participated in the study. The study was therefore discontinued. An update on this study was presented at the International Congress on Neuromuscular Diseases, Naples, Italy, 17-22 July 2010 by Professor Kate Bushby. Details of this presentation is available on www.ptcbio.com Briefly, analysis showed that, on average, patients treated with low-dose ataluren experienced better outcomes on measures of efficacy than patients treated with high-dose ataluren or placebo - this phenomenon is not unique for ataluren and has been observed with other drugs for other diseases. Further analysis of efficacy data is ongoing.

ANTISENSE OLIGONUCLEOTIDE INDUCED EXON SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY

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

33. RESTORING DYSTROPHIN EXPRESSION IN DUCHENNE MUSCULAR DYSTROPHY: A PHASE I/II CLINICAL TRIAL USING AVI-4658
Status: completed
Sponsor: Imperial College London
Funder: Department of Health (DoH)
PI: Prof. Francesco Muntoni
Patients recruited: 8

The primary scope of the trial is to assess efficacy (dystrophin production) and safety of intramuscular administered morpholino oligomer directed against exon 51 (AVI – 4658 PMO). Antisense therapy with the use of antisense oligomers has the potential to restore effectively the production of dystrophin, the defective protein, in >70% of DMD. This could result in increased life expectancy through improved muscle survival and function. Recent scientific research has demonstrated the potential of this technique to skip mutated dystrophin exons, restore the reading frame and generate functional dystrophin protein. Having demonstrated proof-of-principle in human cell culture and animal model studies, we now intend to determine efficacy and safety of this approach to induce dystrophin exon skipping in children with DMD. This study

is aimed at children with Duchenne muscular dystrophy above the age of 10 years with mutations than can be rescued by the skipping of exon 51 [45-50; 47-50; 48-50; 49-50; 50; 52; 52-63].
This work has been completed and outcome data published in the journal *Lancet Neurology (Volume 8, Issue 10, Pages 918 - 928, October 2009)*

34. DOSE-RANGING STUDY OF AVI-4658 TO INDUCE DYSTROPHIN EXPRESSION IN SELECTED DUCHENNE MUSCULAR DYSTROPHY (DMD) PATIENTS – (Systemic study)
Status: Completed
Sponsor: AVI Biopharma
Funder: Medical Research Council (MRC) and AVI Biopharma
PI: Prof. Francesco Muntoni
Patients recruited: 19
This is a safety study of AVI-4658 (a 30-base phosphorodiamidate Morpholino oligomer [PMO]), to skip exon 51 of the dystrophin gene in relevant subjects with DMD.
This is an open-label, two-centre, dose-ranging comparative clinical study of duration twelve weeks.
The objectives of the study are to assess safety and to select the optimum dose that elicits at least 10% de novo dystrophin-positive fibres and dystrophin in a sentinel muscle group after an intravenous AVI-4658 dosing regimen.
A total of up to 16 subjects (ambulatory paediatric males, aged ≥5 and ≤15 years of age) will be enrolled in this study, consisting of four treatment cohorts and four subjects per cohort. It is expected that there will be four treatment arms ranging from 0.5 mg/kg to 4 mg/kg. All subjects will receive 12 weekly intravenous infusions of AVI-4658.
Precedent studies have demonstrate that AVI-4658 might have therapeutic relevance in managing DMD for boys whose frame-shifted dystrophin gene lesion could be restored after excision of exon 51 if sufficient drug is translocated into the nucleus of the afflicted muscle cell.
This trial was conducted in London and Newcastle.
A total of 19 subject (12 at GOSH and 7 at RVI, Newcastle) were recruited and final data is being analysed for submission to regulatory authorities in Europe and the USA. Outcome data were presented at the World Muscle Society, 12-16 October 2010 in Japan and published.
www.thelancet.com *Published online July 25, 2011 DOI:10.1016/SO140-60756-3.*

35. ECULIZUMAB FOR MYASTHENIA GRAVIS
Full Title: A Randomised, Double-Blind, Placebo-controlled, Cross-over, Multicenter Study of Eculizumab in Patients with Generalised Myasthenia Gravis (GMG) who have Moderate to Severe Muscle Weakness Despite Treatment with Immunosupressants
Status: Closed
Sponsor/Funder: Alexion Pharmaceuticals, Inc.
PI: Prof. Dimitri Kullmann
This is a randomized, double-blind, placebo-controlled, cross-over, multicenter study to evaluate the safety and efficacy of eculizumab for the treatment of patients with myasthenia gravis.
Myasthenia gravis (MG) is an acquired autoimmune syndrome caused by the failure of neuromuscular transmission, which results from the binding of autoantibodies to proteins involved in signalling 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.
Wiley Periodicals, Inc. Published online March 2013, (wileyonlinelibrary.com) DOI 10.1002/mus.23839

36. 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: Completed
Sponsor: University College London (UCL)
Funder: Arthritis Research UK and Myositis Support Group
PI: Prof. Michael Hanna
Patients recruited: 12; target 12
Sporadic Inclusion Body Myositis (IBM) is the commonest acquired disease of muscle affecting people aged 50 years and over. This is a progressive and debilitating disease with both muscle weakness and wasting, characteristically of the quadriceps and finger flexors. Over time the condition can lead to severe disability, falls and swallowing impairment. Affected muscle tissue demonstrates inflammation and degeneration.
Arimoclomol is a new compound which acts by enhancing a normal, inbuilt protective cell reaction to stresses. The products of this response are 'Heat Shock Proteins (HSPs) which counteract processes that end up leading to abnormal protein deposition and to damage mediated by inflammation. This proposal involves a multi-centre, double-blind, placebo-controlled parallel study of total duration twelve weeks. This study proposal aims to assess the safety and tolerability of Arimoclomol (100 mg TDS) as compared with placebo over 4 months of treatment in patients with IBM. Recruitment will take place at the National Hospital for Neurology and Neurosurgery and twelve patients will be enrolled.
Manuscript in preparation for publication

37. Investigation of the ability of Otelixizumab to inhibit in vitro antigen-specific T cell responses from Myasthenia Gravis patients
Status: Completed
Sponsor/Funder: GlaxoSmithKline
PI: Prof. Dimitri Kullmann
Patients recruited: 39; target 40
Myasthenia Gravis (MG) is the best understood autoimmune disease (a disease in which the immune system attacks some part of the body). This attack is directed by various parts of the immune system.
There is a continued search for newer drugs that will be of benefit in the treatment of MG. Otelixizumab has been identified as a possible treatment for MG. However before clinical trials can be considered additional information is needed to determine how it interacts with the immune system of patients with MG.
In this study adult patients with MG will be invited to provide blood samples (50 ml) for research purposes. Blood collected from patients will be used for T-cell assay and autoantibody assay development. Patients may be asked to provide a repeat blood sample (additional 50ml) after 46 months following the initial collection to see if T cell activation changes over time. Up to 40

participants will be enrolled in the UK. The study is being sponsored by GlaxoSmithKline group of companies.
For information on recruitment contact Natalie James (natalie.James@uclh.nhs.uk).

38. GSK/Prosensa clinical trial in DMD boys with study drug GSK2402968 (PRO051)
Full Title: A phase II, double-blind, exploratory, parallel-group, placebo-controlled clinical study to assess two dosing regimens of GSK2402968 for efficacy, safety, tolerability and pharmacokinetics in ambulant subjects with Duchenne muscular dystrophy
Status: Completed
Sponsor: GlaxoSmithKline
Funder: GlaxoSmithKline
Newcastle
CI/PI: Prof. Volker Straub
London
PI: Prof. Francesco Muntoni
Recruitment target (UK): 8; Patients recruited (UK): 8
A multicentre trial with this study drug is recruiting DMD boys in UK at the Great Ormond Street Hospital (GOSH), London and at the Royal Victoria Infirmary, Newcastle. It is a Phase lla, double blind, exploratory, parallel clinical trial to assess the optimal dose of GSK2402968 for safety, tolerability and efficacy, in ambulant patients with DMD. This study is designed to explore efficacy and safety of GSK2402968 given as a continuous regimen and an intermittent regimen over 24 and 48 weeks.
Objective(s)
Primary objective:
• To assess the efficacy of 2 different dosing regimens of subcutaneous GSK2402968 administered over 24 weeks in ambulant subjects with DMD.
Secondary objectives:
• To assess the safety and tolerability of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.
• To assess the PK of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.
• To assess long term efficacy of 2 different dosing regimens of subcutaneous GSK2402968 administered over 48 weeks in ambulant subjects with DMD.
The study aims to randomise 54 subjects. There will be 2 parallel cohorts. Each cohort will include 16 subjects on GSK2402968 and 8 subjects on matched placebo (2:1 ratio). Further information about this study can be obtained from the MRC Centre Clinical Trials Coordinator on 020 7905 2639.
In the process of being published

39. THERAPEUTIC TRIAL OF LITHIUM CARBONATE IN MND/ALS (LiCALS)
Full title: A double-blind, randomised, placebo controlled trial of lithium carbonate in patients with amyotrophic lateral sclerosis.
Status: Completed
Sponsor: University College London Hospitals NHS Foundation Trust
Start date: June 2009
Funder: Motor Neurone Disease Association, and NIHR
UCL PI: Dr Richard Orrell
Patients recruited: 22, target: open-ended
Recent research suggested that lithium carbonate may be effective in lowering the progression of MND/ALS. Lithium may protect motor neurons through a range of mechanisms, including improving the transport of proteins along the motor neuron, improving the transport of

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

40. LiCALS Open Label Extension
Full title: LiCALS open label extension trial of lithium carbonate in amytrophic lateral sclerosis
Status: Completed
Sponsor: University College London Hospitals NHS Foundation Trust
Start date: March 2011
Funder: Motor Neurone Disease Association, and NIHR
UCL PI: Dr Richard Orrell
Patients recruited: 3 of 8 recruited
This is an open label extension study for those who have completed the randomised double blind trial of lithium carbonate in ALS. The objective is to obtain further evidence of the safety of lithium carbonate in doses achieving levels of 0.4-0.8 mmol/l. *Results in Press*

41. GSK1223249 in MND/ALS (the Nogo-A study)
Full title: A Phase I, multi-centres, randomized, placebo-controlled, double-blind, single and repeat dose escalation of a drug to treat ALS
Status: Completed
Sponsor: Royal Free Hampstead NHS Trust
Start date: September 2010
Funder: GlaxoSmithKline
UCL PI: Dr Richard Orrell
Patients recruited: 2, target: 2
GSK 1223249 is a new drug developed by GlaxoSmithKline, that targets a protein called Neurite Outgrowth Inhibitor (Nogo-A), which impairs neurone regeneration. There is evidence of increased Nogo-A, which impairs neuron regeneration, in muscle of people with MND/ALS. By blocking the effect of Nogo-A, GSK1223249 may be an effective treatment for the disease. GSK1223249 delays symptom onset and prolongs survival in a mouse model of MND/ALS. The trial will provide safety and tolerability information, together with biomarker and functional information. This may leader to further trials to assess effectiveness. The study includes an infusion of the drug (or placebo), with a muscle biopsy taken before and following the infusion, together with other monitoring assessments. For further information please contact Dr Richard Orrell (r.orrell@ucl.ac.uk). *Results in Press*

42. HYP HOP: DICHLORPHENAMIDE vs. PLACEBO FOR PERIODIC PARALYSIS
Full Title: Double-blind, placebo-controlled, parallel group, phase III study comparing dichlorphenamide vs. placebo for the treatment of periodic paralysis
Status: Completed
Sponsor: University Rochester
Funder: National Institutes of Health (NIH - USA)
PI: Prof. Michael Hanna
Patients recruited:14; target 40
This is a phase III trial into Periodic Paralysis. This proposal involves a multi-center, 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.
Manuscrip in preparation

43. Phase II, multicenter, randomized, adaptive, double-blind, placebo controlled Study to assess Safety and Efficacy of Olesoxime (TRO19622) in 3-25 year old Spinal Muscular Atrophy (SMA) patients
Status: Completed
Sponsor: TROPHOS
Funder: Association Francaise contre les Myopathies
Newcastle
PIs: Prof. Hanns Lochmuller, Dr Helen Roper
London
PI: Prof. Francesco Muntoni,
Recruitment target (UK): 30; GOSH: 10, Newcastle: 3
The UCL Institute of Child Health and Great Ormond Street Hospital for Children (London), Birmingham Heartlands Hospital, and Newcastle upon Tyne Hospitals Royal Victoria Infirmary have been invited to collaborate in this phase II clinical trial in non-ambulant patients with SMA II and III with a documented homozygous absence of SMN1 exon 7 and/or deletion and mutation on the other allele. This is a multicentre, double-blind, randomized, placebo-controlled study in patients with SMA type 2 or non-ambulant type 3. The study will be conducted in multiple centres across Europe and will be sponsored by Trophos (a biopharmaceutical company based in France) and funded by AFM (Association francaise contre les myopathies). The aim is to assess efficacy, futility, safety and tolerability of a new drug called olesoxime. This is a neuroprotective drug that acts by interacting with protein components of the mitochondrial permeability transition pore (mPTP), preventing the release of apoptotic factors and in turn neuronic death. Olesoxime has displayed an excellent safety profile and has been well tolerated in phase I clinical trials in healthy subjects. For each participant, this phase II study will involve a 4 week screening period followed by a 24 month (104 week) treatment period. Following screening procedures and confirmation of eligibility, subjects will be randomised to receive either olesoxime or placebo in a 2:1 ratio. Olesoxime (or matched placebo) will be taken daily with evening meal as a liquid formulation at a dose of 10mg/kg. 150 subjects in total will be recruited, with a target of 30 patients in the UK. Recruitment is planned to be completed in 6 months. It is possible a dose adjustment may be made once 45 patients across Europe have been received study drug for 3 months based on a review by a designated independent Data Monitoring Committee. The patients to be recruited should be at least 3 years of age but younger than 26 years at the time of enrolment, with the age of onset of symptoms to be at 3 years of age or younger. They should not be taking any medication intended for the treatment of SMA within 30 days prior to being enrolled on the study. Eligible patients can be taking oral salbutamol as long as this has been commenced at least six months prior to enrolment on the study and remains at a stable dose during the study period. Participation in another investigational drug or therapy study within 3 months of enrolment is an exclusion criterion, as well as a hypersensitivity to sesame oil and use of medications that could interfere with olesoxime absorption (including cholesteramine, fibrates, fish-oils, niacin, phytosterols and ezetimibe).
Further information about this study can be obtained from the Clinical Trials Coordinator on 020 7905 2639.
Data in analysis

44. The PATH Study
Full title: Randomized, multicenter, double-blind, placebo-controlled, parallel-group phase III study to investigate the efficacy, safety and tolerability of 2 different doses

of Igpro20 (subcutaneous immunoglobulin) for the treatment of chronic inflammatory demyelinating polyneuropathy (CIDP) – the Path Study
Status: completed
Sponsor: CSL Behring
PI: Dr Michael Lunn
Patient target: 5; recruited 6
CIDP is an acquired neurological, demyelinating neuropathy with an assumed autoimmune-mediated pathogenesis. Due to its heterogeneous presentation and the limitations in the individual diagnosis procedures (clinical, serologic, and electrophysiological), the diagnosis relies on findings from multiple modalities. The probable autoimmune nature of the condition is most strongly suggested by response to immunotherapies such as intravenous immunoglobulins (IVIGs), plasmapheresis (PE), and corticosteroids.
In addition, despite less definitive published evidence of efficacy, corticosteroids are also considered as first-line therapy because of their long history of use.
Apart from IVIGs, there are currently no other medications approved for the treatment of CIDP; however experimental use of azathioprine, mycophenolate mofetil, methotrexate and cyclosporine are common and whilst there are also emerging reports of the use of B Lymphocyte antigen CD20 and anti-complement monoclonal antibody therapies, efficacy has not been established for any of these agents.
This is a prospective, multicenter, randomised, double-blind, placebo-controlled, parallel-group 3-arm study to investigate 2 different doses of SCIG IgPro20 compared to SC (subcutaneous) placebo for maintenance treatment of subjects with CIDP. Subjects on IVIG maintenance therapy experiencing CIDP relapse during an IVIG Withdrawal period will be administered the IVIG IgPro10 (1 loading dose and 3 or 4 maintenance doses every 3 weeks) during an IVIG Re-stabilization Period. Subjects with improved and maintained INCAT score at the last 2 assessments in the IVIG Re-stabilization Period will be randomised to 1 of 2 Igpro20 doses (0.2 or 0.4 g/kg body weight) or placebo during the SC Treatment Period.
IgPro20 is a ready-to-use formulation of human IgG with ≥98% purity for subcutaneous (SC) administration. Igpro20 is approved in the United States of America (US), in the EU, in Switzerland and Canada under the brand name Hizentra® for SC application in primary immune deficiency syndromes and is also under review by other regulatory agencies for use in primary and secondary immunodeficiencies.
IgPro10 is a ready-to-use liquid formulation of polyvalent IgG for intravenous (IV) application approved and marketed in several countries including the European Union (EU) and the US for use in primary immunodeficiency (PID) syndromes and for immune thrombocytopenic purpura (ITP). In the EU, IgPro10 is further approved for other conditions associated with immunodeficiencies resulting in the need for replacement therapy and in Guillian-Barre Syndrome (GBS) where IVIG is thought to have immunomodulatory effects on the peripheral nervous system. For the treatment of GBS and CIDP, a similar mode of action is assumed.
IgPro10 is currently under investigation in a confirmatory phase III study in subjects with CIDP.
Several randomised clinical studies have demonstrated the clinical efficacy and safety of using IVIGs to treat CIDP.
IVIGs requires subjects to visit a clinic or hospital for 1 to 5 days on a regular basis, usually every 2 to 6 weeks. This study is being conducted to provide SCIG as an alternative treatment option for CIDP that allows subject (or their caregiver) to self-administer the product in the home setting.

45. SMT C1100 – A Phase 1, Open-label, Single and Multiple Oral Dose, Safety, Tolerability and Pharmacokinetic Study in Paediatric Patients with Duchenne Muscular Dystrophy
Status: Closed

Sponsor & Funder: Summit
PI: Prof. Francesco Muntoni
Patients Recruited: 4
This will be an open-label, single and multiple oral dose study. Up to 12 patients with DMD will be enrolled onto the study. Primary Objective is to determine the safety and tolerability of single and multiple oral doses of SMT C1100 in patients with Duchenne Muscular Dystrophy (DMD). Secondary Objectives are to determine the single and multiple oral dose pharmacokinetics of SMT C1100 and its metabolites in patients with DMD.

SMT C1100 is the first in a new pharmacological class of orally available small molecules that act to modulate transcriptional control of utrophin. SMT C1100 is being developed with the potential to treat DMD independent of the dystrophin mutation, by maintaining production of utrophin to compensate, at least in part, for the loss of the dystrophin protein. Outcomes from non-clinical pharmacodynamic studies indicate that SMT C1100 increases utrophin mRNA and protein levels and improves muscle structure and function.

Natural History – Longitudinal Studies

Set-up Phase

46. LEMS Disease Registry – UK Proposal
Status: set-up
Sponsor: BioMarin Europe Ltd
PI: Prof. Michael Hanna
Patients target: 10 from the NHNN
The LEMS registry is a voluntary multi-centre, multinational, observational program for patients with LEMS disease and is intended to track the routine clinical outcomes of patients with LEMS over time.
The purpose of the LEMS registry is to collect additional data on the long term safety and efficacy of Firdapse for patients who have been prescribed Firdapse by their treating physician. The registry will also track the use of treatment for LEMS including drugs other than Firdapse. The data collected by the registry are intended to enable better characterisation of the natural history of LEMS.
As this is an observational (non-interventional) programme no experimental treatments or assessments are involved, it is up to the treating physician to determine the actual frequency of assessments according to the patients' individual need for medical care and routine follow-up. All patients with a confirmed diagnosis of LEMS is eligible to participate in this programme, confirmation can be by abnormal Electromyogram (EMG) or positive result for Voltage Gated Calcium Channel (VGCC) antibodies, however patients cannot be participating in any other study with Firdapse.

47. Prospective. Longitudinal Study of the Natural History and functional status of patients with MyoTubular Myopathy (NatHis-MTM)
Status: set-up
Sponsor: Institute of Myology
PI: Prof. Francesco Muntoni
Patients target: 6-8
Centronuclear myopathy (CNM) is an inherited neuromuscular disorder. It is a group of rare congenital myopathies characterized by the presence of hypotrophic myofibers with centrally placed nuclei on muscle biopsies. CNM exists in 3 forms: i) X-linked recessive (OMIM 310400), ii) autosomal dominant (OMIM 160150) and iii) autosomal recessive form (OMIM 255200). This study will be a multicentre international study in Europe and USA. Presently, there is no effective therapy to treat the muscle weakness in XLMTM patients. Current treatments include mainly

respiratory, feeding and orthopedic management. These treatments improve muscular function, quality of life and longevity but do not directly target the disease mechanism. Primary objective of this study is to characterize the disease course in MTM patients using standardized evaluations. Secondary objectives are to identify prognostic variables of the disease; to identify the best outcome measure(s) for future treatment studies; to assess the immune response against AAV.

48. Becker Muscular Dystrophy - A Natural History Study to Predict Efficacy of Exon Skipping
Sponsor: CINRG
Newcastle:
CI: Prof. Kate Bushby
PI: Dr Michela Guglieri
This is a multi-center natural history study that will be conducted at CINRG sites and affiliated centers. Following a baseline evaluation, participants will have three follow-up visits over a three-year period. We will characterize the BMD phenotype, and correlate specific abnormal dystrophin proteins with the range of clinical outcomes.

Open Studies

49. FSHD registry
Status: Ongoing
PI: Prof. Hanns Lochmuller
The UK FSHD Patient Registry is a national registry for all people affected by facioscapulohumeral dystrophy living in England, Scotland, Wales and Northern Ireland. It is a patient driven online registry launched in May 2013. The main purpose of the registry is to facilitate and accelerate the recruitment into clinical research while also providing a resource to help plan and design clinical trials. In addition to collecting an internationally agreed dataset the registry is a platform for additional research questionnaires collecting information about pain, quality of life and scapular fixation. The registry is funded by the Muscular Dystrophy Campaign and supported by the TREAT-NMD Alliance.

50. CMT: A NATURAL HISTORY STUDY
Full Title: Charcot-Marie-Tooth Disease and related disorders: A Natural History Study
Status: Open to Recruitment
Sponsor: University College London Hospitals
Funder: National Institutes of Health (NIH – USA)
CI: Prof. Mary M. Reilly/ PI: Prof. Francesco Muntoni
Patients recruited: NHNN 772; GOSH 71; target (UK) 650
Charcot-Marie-Tooth Disease (CMT) and related disorders (distal hereditary motor neuropathy (dHMN) and hereditary sensory and autonomic neuropathy (HSAN)) are a clinically and genetically heterogenous group of disorders affecting approximately 1 in 2500 people.
People with this condition present with upper and lower limb weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles. Despite the clinical similarities among patients with CMT the group is genetically heterogeneous. Advances have been made in identifying the genes that cause CMT and the molecular organisation of the peripheral nervous system (PNS) nevertheless the optimal management and treatment of the different variants of this disorder is not known and moreover natural history data is lacking for most forms of inherited neuropathies.
This is a 5 year study that will be conducted by four centres in United States and two centres in the UK (National Hospital for Neurology and Neurosurgery and Great Ormond Street Hospital). The aim of the project is to fully characterise the features of different types of CMT and the longitudinal progression of the disease. The data will also be used to establish clinical relevant

endpoints for use in therapeutic trials. The identification and genetic characterisation of patients will facilitate the recruitment of participants for future therapeutic trials. Ultimately the information gained with this study will lead to the improvement in the treatment and management of CMT.
The study is also seeking to establish an appropriate paediatric impairment scoring method for CMT and establish a database for the inherited neuropathies. The study will include both adult and paediatric patients. Evaluations will consist of a neurological history and examination, nerve conduction velocity (NCV) study and in some selected cases skin biopsy.
This is a NIH funded study. At least fifty patients will be enrolled at the National Hospital for Neurology and Great Ormond Street Hospital.
For more information about the study please contact Dr. Matilde Laura at m.laura@ion.ucl.ac.uk.

51. Natural History study of Hereditary Sensory Neuropathy type 1 secondary to SPTLC1 and SPTLC2 mutations
Status: Open to Recruitment
Sponsor: University College London Hospitals
PI: Prof. Mary M. Reilly
Patients recruited: 32
Hereditary Sensory Neuropathy Type I secondary to SPTLC1 and 2 mutations is the commonest of the Hereditary Sensory Neuropathies. It is a slowly progressive neuropathy leading to profound loss of sensation especially pain and temperature sensation with variable but often severe motor involvement. Most patients have sensory complications such as recurrent ulcers; osteomyelitis and amputations are common. Over time, there is considerable disability requiring extensive carer support.
There is emerging evidence for the use of serine as a potential treatment option. This rapid progress has led to the possibility of a clinical therapeutic trial of serine in our UK HSN1 population.

A longitudinal study is now underway to determine the best way of measuring diseases progression in this condition which can be used in a clinical trial. We have a unique population within the United Kingdom where all the SPTCL1 patients (56) have a common mutation (C133W). Despite this, there is significant heterogeneity in the phenotype. A variety of assessment methods to cover the spectrum of deficits noted in this condition will be performed and repeated after a year. These include: CMT Neuropathy Score, comprehensive neurophysiological assessment, Quantitative Sensory Testing (DFNS protocol), muscle MRI studies of the thighs and calves, machine myometry, analysis of plasma DSB levels, upper thigh skin biopsy (epidermal nerve fibre density measurements) and patient questionnaires (SF36 and NPSI).

For more information contact: Dr Maiya Kugathasan, u.kugathasan@ucl.ac.uk

52. MITOCHONDRIAL DISEASE COHORT (New Title MRC Centre Mitochondrial Disease Patient Cohort: A Natural History Study and Patient Registry)
Status: Ongoing
Sponsor: Newcastle Upon Tyne Hospitals NHS Foundation
Funder: MRC
PIs: Dr R McFarland, Prof. Michael Hanna, Prof. D Turnbull
Total target 1500
Recruitment to date 1150
Newcastle: 548
UCL: 340
Oxford: 115
GOSH: 37
Satellites: 110

Mitochondrial disease presents a huge challenge to patients and doctors because no effective treatment is available. The extremely diverse phenotypic presentation of mitochondrial disease has previously limited cohort development. Consequently, despite a minimum prevalence of 9.2/1000000, there are currently no large cohorts of patients with genetically/biochemically confirmed mitochondrial disease anywhere in the world. To enable us to gain adequate, credible results from phase III clinical trials, we developed (with funding secured by the MRC) the MitoCohort Patient Database. The cohort comprises symptomatic adults (including adults lacking capacity) and children, in whom a mitochondrial disease phenotype and biochemical deficiency and/or genetic mutation have been confirmed. Asymptomatic individuals who have requested genotyping and proved positive are also included. The Mito Cohort recruits patients on a national level with sites throughout UK mainland. Data collected can be accessed by applying to the Mitochondrial Disease Oversight Committee which includes an ethicist and patient representative. Applications are considered by the committee and if approved anonymised data is released via the team's research associate or research nurse.

For information on the status of recruitment or to apply to use collected data please contact or Julia.Maddison@newcastle.ac.uk and
Dr Yi Ng (Newcastle) yi.ng@ncl.ac.uk

53. THE NATURAL HISTORY OF INCLUSION BODY MYOSITIS (IBM Net)
Status: Open to Recruitment
Sponsor: University College Hospitals
Funder: MDC
PIs: Dr Matt Parton/ Prof. Michael Hanna
Target 120-150; recruited 70
Inclusion body myositis (IBM) is probably the commonest muscle disease beginning in those aged over 50. It leads to progressive disability with, classically, a characteristic pattern of muscle involvement. However it is poorly understood: its cause is unknown, there is no conclusive diagnostic test and it has no treatment. Furthermore, information on the pattern and prognosis of IBM is more based on anecdote from clinical experience, rather than firm fact. The largest published series of data on the natural history of the illness followed only eleven patients for six months.
The current project seeks to better characterise IBM by gathering clinical data from as many cases as possible.
Serial standardised assessment (annually for five years) will chart disease progression and so both expand and strengthen knowledge of the natural history of the illness. Furthermore, establishment of a cohort of reliably-defined cases will build a valuable resource that could potentially form the starting-point for future studies.
For information on the status of recruitment please contact Dr. Pedro Machado at p.machado@ucl.ac.uk
Neuromuscul Disord. 2013 May;23(5):404-12. doi:10.1016/j.nmd.2013.02.010. Epub 2013 Mar 11. PubMed PMID: 23489664

54. Kennedy's Disease – Study and Register
Status: Open
Sponsor: UCLH
PI: Prof. Michael Hanna
Patients recruited: 56
The primary purpose of this study is to create a national register of patients with Kennedy's Disease (spinal and bulbar muscular atrophy) with a view to facilitating research into the disorder. In particular, we aim to systematically characterise diagnostic features of the disorder and their natural history and attempt to estimate the incidence and prevalence of Kennedy's disease in the United Kingdom. Furthermore we intend to assess the experience of patients with

regard to specialist neurological, endocrinological and clinical genetic care and, by so doing, to establish best practice guidelines for the diagnosis and management of this disorder.
Kennedy's disease was first described as a separate entity in a series of 9 males in 2 families (Kennedy et al., 1968) and prior to this was not distinguished from adult-onset forms of spinal muscular atrophy. Kennedy described the disorder as being X-linked on the basis of his pedigrees, and the causative mutation in the X-chromosome was tracked in 1991 to the Androgen Receptor. The disease is caused by the expansion of an intragenic CAG triplet repeat in exon 1 of the gene which is translated into a polyglutamine segment in the AR protein. As such Kennedy's disease became the first in a series of 9 disorders now known to be caused by such expanded polyglutamine repeats (the others being Huntington's disease, dentato-rubral pallido-luysian atrophy and spino cerebellar ataxias).
The earliest clinical features are androgen insensitivity, postural hand tremor and muscle pains with subsequent development of motor neuropathy, bulbar signs and symptoms and a distal sensory neuropathy which is usually subclinical.
As the prognoses of these two conditions are very different it is clearly important that these patients are correctly identified and managed. Furthermore, patients with Kennedy's disease have an additional set of endocrine and metabolic problems over and above the more well-defined neurological deficits. The endocrine and metabolic aspects of the disorder in particular are poorly characterised and their relationship to the genotype is controversial. Their implications for patients in terms of morbidity has also not been investigated.
The study proposes to, if possible, interview and examine patients directly and attempt to gain a time course of the development of individual symptoms and signs. Wherever molecular genetic confirmation of the diagnosis has not already been performed by the referring hospital this will be performed by standard PCR methods with the prior explicit consent of the patient and the referring neurologist. Creatine kinase, and endocrine function (testosterone, luteinising hormone and sex-hormone binding globulin levels) will be assessed from blood samples by standard techniques. Pedigrees for the patient's families will be obtained from the hospital notes, or from direct interview where this has been possible.
By creating a register of the United Kingdom's Kennedy's population we hope to obtain clear evidence of phenotype-genotype correlation and, over time, establish relationships with disease severity and prognosis.
For further information contact: Dr Pietro.Fratta, p.fratta@prion.ucl.ac.uk

55. Investigation of Human Neurological Ion Channel Disorders
Status: ongoing
Sponsor: University College London Hospitals
PI: Prof.. Michael Hanna
Recruited: 56
Ion channels are membrane bound proteins that allow the flux of charged ions across cell membrane in excitable tissue including muscle and nerve. These ion channels are usually specific for a particular ion e.g. potassium or calcium. An increasing number of inherited and acquired neurological diseases are attributed to disorders of ion channels. The 'channelopathies' include non-dystrophic myotonia, periodic paralysis, episodic ataxias types 1 and 2 (EA1 and EA2), familial hemiplegic migraine types 1 and 3 (FHM1 and 3) and some forms of epilepsy. The mechanisms by which ion channel dysfunction causes disease are incompletely understood, but the genetic channelopathies have provided unique insights into because the properties of mutant channels can be studied with great precision with biophysical methods.
Although each of the known channelopathies is rare, there is considerable circumstantial evidence that genetic variability in ion channels plays a major role in idiopathic epilepsy and migraine. These disorders, which are characterised by normal brain development and function punctuated by episodes of abnormal excitability, show strong heritability although typically do

not respect Mendelian patterns of inheritance. They represent an important disease burden to society. It remains to be determined whether such disorders are caused by many, individually rare genetic variants or by a few common polymorphisms affecting ion channel splicing, assembly, trafficking or function.
Several intense programmes of research are underway to identify genetic susceptibility factors in epilepsy and other paroxysmal neurological diseases. The purpose of this proposal however, is to take a complementary approach to gain an insight into the consequences of genetic variability, focusing primarily on KCNA1, mutations of which cause EA1(5) and CACNA1A mutations which cause EA2(6), and to document these at all levels from molecular biophysics to cellular excitability studied in individuals harbouring the mutations. Only by understanding the degree to which variability of ion channel properties can be tolerated by the organism, or conversely affect neuronal excitability in a detectable manner, will we be able to interpret the functional impact of coding polymorphisms that are staring to be reported in population studies.

This research project aims to consolidate and expand on previous work by collating clinical data and continuing to sequence candidate genes in patients suspected to have ion channel disorders, particularly in Episodic Ataxia Type 1 (KCNA1 gene) and Episodic Ataxia type 2 (CACNA1A gene). As the project progresses it is possible that further candidate genes will be identified and we will sequence these also. In vitro expression of new mutations will be performed in order to further study how these genetic mutations result in channel dysfunction.

56. AFM Natural History Study
Full Title: Outcome measures in Duchenne Muscular Dystrophy: A Natural History Study
Status: Ongoing
Sponsor: UCL Institute of Child Health
Funder: AFM
London
PI: Prof. Francesco Muntoni
Newcastle
PI: Prof. Kate Bushby
Patients recruited: 20 (GOSH) 20 (Newcastle)
Description: To document with quantified measurements the natural history of Duchenne Muscular Dystrophy. Several validated tools will be used to describe motor, orthopaedic and respiratory functions, quality of life and blood parameters along a 4 years follow-up study in ambulant and non-ambulant patients.

Primary objective is to document with quantified measurements the natural history of Duchenne Muscular Dystrophy. Several validated tools will be used to describe motor, orthopaedic and respiratory functions, quality of life and blood parameters along a 4 years follow-up study in ambulant and non-ambulant patients.
Secondary objectives are specific tests to ambulant and non-ambulant patients will be performed. All these tests should determine the most sensitive outcome measures to use in the assessment of efficacy of future therapies. This prospective longitudinal natural history study will be performed in two cohorts of patients with DMD according to their level of functional motor ability (ambulant/non-ambulant). Inclusion criteria and methods will be different in the two cohorts and will be described separately.

57. Using Next Generation Sequencing to Unravel the Pathogenesis of Sporadic Inclusion Body Myositis (IBM) – The International IBM Consortium Genetic Study
Status: Ongoing
Sponsor:
Funder: MRC
London
CI: Prof. Michael Hanna
Newcastle

PI: Prof. Volker Straub
Patient target: 400
Recruited: 73 (NHNN); 20 (Newcastle)
The primary pathogenesis of IBM is not determined, although in IBM the aggregated proteins are in muscle tissue, many such as tau, alpha synuclein, TDP-43, beta amyloid and the prion protein are implicated in neurodegeneration. It is possible that the defective processes that lead to the formation of these abnormal protein deposits are likely to have important implications for many neurological disorders.
The vast majority of IBM is sporadic but there is significant evidence to suggest that genetic factors are important in IBM; these include the compact age at onset, insidious progression, clinical and pathological features, infrequent occurrence in twins, siblings and families. There have been several Mendelian genes identified in families with IBM phenotypes but these are rare. To investigate the pathogenesis of IBM further requires a genomic approach on large numbers of defined cases.
We will establish an international collaboration to collect IBM patient DNA and detailed clinical information to facilitate IBM research - The International IBM Consortium Genetic Study (IIBMCGS).
The number of IBM cases worldwide is not large enough for an effective genome wide association study (GWAS) but using next generation exome sequencing we can identify rare coding variants and high-risk genome wide variants in IBM. This technique has been used effectively in the identification of mutations that cause Mendelian disorders and more recently found significant rare coding variants in type I diabetes and autism.
In this proposal we wish to employ exome sequencing to analyse 200 IBM cases and 200 normal muscle controls. We expect to identify a number of IBM rare variants that cluster in disease associated genes. We plan to replicate these findings in a further 700 IBM and 2200 controls. These data will be made publicly available (anonymously) to allow comparison with other muscle disorders and neurodegenerative conditions.
Orphanet J Rare Dis. 2014 Jun 19;9:88. doi:10.1186/1750-1172-9-88. PubMed PMID: 24948216; PMC4071018.

58. Hereditary Inclusion Body Myopathy-Patient Monitoring Program (HIBM-PMP): A Registry and Prospective Natural History Study to Assess HIBM Disease
Status: Recruiting
Sponsor: Ultragenyx
Funder: Ultragenyx
CI/PI: Prof. Hanns Lochmuller
Patients recruited: 23
Target: 15-20
HIBM is a severe progressive myopathy that typically presents in early adulthood as weakness in the distal muscles of the lower extremities and progresses proximally, leading to a loss of muscle strength and function, and ultimately a wheelchair-bound state. The rate of progression is gradual and variable over the course of 10-20 years or longer. There is a need to better understand the disease-specific features of HIBM to heighten disease awareness; facilitate early diagnosis; identify patients; expand knowledge of the clinical presentation, progression and variation of the disease; identify and validate biomarkers and other efficacy measures; inform on the design and interpretation of clinical studies of investigational products; and eventually to optimize patient management.

Up to 10 centers in North America, the European Union (EU), and the Middle East will participate in the HIBM prospective observational study (hereto referred to as the HIBM Natural History Study).
HIBM Disease Registry subjects may or may not be associated with a Study Site. Some disease registry subjects may enter only self-reported data and will not be associated with a clinical site. Other disease registry subjects who are in the same country as a natural history site may choose to have their data confirmed by one of those centers or opt-in to the HIBM Natural History Study.

The main objective of this program is to better understand HIBM.
The specific HIBM Disease Registry's objectives are to:
• Identify HIBM patients worldwide.
• Promote awareness and facilitate diagnosis of HIBM disease in the neuromuscular field.
• Obtain an assessment of the medical history, clinical presentation and progression of disease in HIBM patients and provide a connection for subjects to the broader HIBM community and associated programs.
• Provide customized information to subjects and their physicians that desire information on their disease status and progression.

The specific HIBM Natural History Study's objectives are to:
• Characterize HIBM disease presentation and progression over time using relevant clinical assessments of muscle strength and function.
• Obtain information to better characterize quality of life and understand the timing of significant life changing events in HIBM patients using patient-reported outcomes.

59. Full title: Prospective evaluation of gastrostomy in MND (PROGAS). Prospective evaluation of gastrostomy in MND (PROGAS).
Status: Ongoing
Sponsor: Royal Free London NHS Foundation Trust
Start date: 2011
Funder: Motor Neurone Disease Association / South Yorkshire CLRN
UCL PI: Dr Richard Orrell
Patients recruited: 6, target: open-ended
Difficulty in swallowing is a common problem in patients with MND. Patients with severe swallowing difficulty experience malnutrition, dehydration, choking and an increased risk of chest infections. Long-term nutritional support of patients with severe swallowing difficulty can be achieved by placing a feeding tube, known as a gastrostomy, directly into the stomach. However, the current practice of gastrostomy feeding is largely based on consensus and expert opinion rather than the outcomes of appropriately designed trials. Currently gastrostomy technique and timing of insertion within the disease course vary throughout the UK. There is a lack of evidence to suggest what the optimal timing for gastrostomy is, or which method is most appropriate. In addition, although gastrostomy is routinely performed, the benefits, such as improved survival and quality of life following gastrostomy, have not been proven. The main aim of this study is to develop evidence-based guidelines for gastrostomy use in patients with MND. Patients and carers will be recruited at the participating MND Centres around the UK. Questionnaires will be used to assess the safety, complications and benefits of the differing timings and methods of gastrostomy insertion. The results of this work will translate into the development of guidelines, which will optimise the benefit, and the patient and carer experience of gastrostomy. The principles will be readily applicable to patients with severe swallowing problems who are eligible for gastrostomy insertion due to other neurological diseases

60. THERAPEUTIC TRIAL OF diaphragmatic pacing IN MND/ALS (DiPALS)
Full title: A randomised controlled trial in patients with respiratory muscle weakness due to motor neurone disease of the NeuRx RA/4 Diaphragm Pacing System
Status: Ongoing
Sponsor: Royal Free London NHS Foundation Trust
Start date: March 2013
Funder: NIHR Health Technology Assessment Programme / Motor Neurone Disease Association / Department of Health subvention funding
UCL PI: Dr Richard Orrell
Patients target: 4 plus
Non Invasive Ventilation (NIV) therapy is the current standard treatment to help allow patients with MND/ALS to breathe. Patients wear a face mask over their nose or mouth or both and as they breathe in, the machine gives an extra push of air to support the patient's weak breathing muscles, enabling a bigger deeper breath. Some MND patients do not tolerate NIV due to the type of mask they have. During the day problems with using NIV include issues like claustrophobia, feeding and communication. Eventually respiratory muscle weakness will

progress to a point at which intermittent/overnight NIV is ineffective. Diaphragm pacing (DP) is a means of increasing the strength of the main breathing muscle. The NeuRx RA/4 Diaphragm Pacing System has been developed for patients who are unable to control their diaphragms because of stable high spinal cord injuries or because they have a neuromuscular disease such as MND. The pacing wires are inserted into the diaphragm muscle during a small operation and are connected to a small portable box that the patient can easily carry about. The proposed study will assess if treatment with DP prolongs life and maintains quality of life when given in addition to current standard care with NIV. 108 patients will be recruited to the study in up to 10 NHS hospitals in the UK. Patients will be randomised to either have NIV or receive DP in addition to NIV. Study participants will be required to complete outcome measures at 5 follow up time points (2, 3, 6, 9 and 12 months). Patients in the DP group will have additional visits for surgery and a 1 week post operative follow up. 12 patients (and their carers) from the DP group will also be asked to complete 2 qualitative interviews.

61. BIOMARKER STUDIES IN MND/ALS
Full title: Characterisation of a panel of disease biomarkers in peripheral blood from individuals with motor neuron disease
Sponsor: University College London Hospitals NHS Foundation Trust
Start date: May 2009
Funder: Motor Neurone Disease Association
UCL PI: Dr Richard Orrell
Motor neuron disease (MND) is an adult-onset neurodegenerative diseases and one of the commonest neuromuscular disorders. The speed of progression of MND varies among individuals and the condition can develop with different clinical manifestations. Currently, there are no blood tests that could help us to predict the speed of progression of the disease and the likely clinical manifestations (e.g. predominant involvement of speech and swallowing or of the limb muscles). We are testing specific disease biomarkers in the blood. To assess change over time, a blood sample is taken every 3 months. The sample has to be carefully processed as soon as it is taken to preserve the quality of the blood contents. We are studying a range of blood constituents including proteins, DNA and RNA. From some participants we also collect samples of cerebrospinal fluid. If repeated samples are not possible, a single sample of blood for DNA studies is also helpful. We also examine samples from participants without MND/ALS, and individuals with similar but unrelated neuromuscular conditions. Parallel studies of biomarkers in an animal model of ALS are informing our choice of biomarkers. The study is in collaboration with Queen Mary University of London, and other participating centres.

62. International Guillain-Barre' Syndrome (GBS) Outcome Study - IGOS
Status: Open
Sponsor: Glasgow University
Funder: Wellcome Trust/GBS Support group
PI: Dr Lunn
Patients target: 10 from the NHNN
Despite partially effective forms of treatment, outcome in patients with Guillain-Barre' syndrome (GBS) has not improved in the last two decades. At present about 10 to 20% of patients remain severely disabled and about 5% die. One explanation for this stagnation is the highly variable clinical course of GBS. Determinants of disease progression and recovery in GBS are still poorly understood. GBS may consist of distinct pathogenic subgroups, in which disease onset and progression is influenced by different types of preceding infections, anti-neural antibodies and genetic polymorphisms. Optimal treatment of individual patients may depend on the pathogenesis and clinical severity.
The international GBS Outcome Study (IGOS) aims to identify clinical and biological determinants of disease progression and recovery in GBS. This information will be used to understand the diversity in clinical presentation and response to treatment of GBS and to develop new prognostic models to predict the clinical course and outcome in individual patients.

IGOS is a prospective observational international multi-centre study including at least 1000 patients with GBS or variants of GBS, including the Miller Fisher syndrome (MFS) and overlap syndromes. The study has a follow-up of one year.
The aim is to obtain a detailed and standardised database on clinic features, treatment, and diagnostic electrophysiology, and collect a biobank with serum samples and DNA at specific visits.
There is an option to collect cerebrospinal fluid (CSF) during routine diagnostic work-up for proteomic studies, and to conduct an extended follow-up of two and three years. Additional studies may be added in the future.
For further details please contact Dr Lunn, Michael.lunn@uclh.nhs.uk

63. Identification of disease susceptibility genes associated with development and clinical characteristics of primary inflammatory muscle diseases, PM, DM and IBM
Status: Ongoing
Sponsor: University of Manchester
Funder: ARC
PI: Isenberg
PM, DM and IBM are a subset of inflammatory muscle disorders of unknown cause, currently classified under the umbrella term of idiopathic inflammatory myopathies (IIM). PM, DM and IBM are characterised by skeletal muscle inflammation and progressive muscle weakness, which can be debilitating and chronic in nature (occasionally fatal). Steroid and immuno-suppressive treatments are often only partially effective at reducing symptoms, and toxic side effects also limit their usefulness.
The cause of muscle inflammation in PM, DM and IBM is unknown. There is, however, increasing evidence that genetic factors, such as the polymorphisms around the complex HLA molecules, as well as certain inflammatory cytokines, are intimately involved in both the development and expression (in terms of disease severity and organs targeted for damage) of these conditions. Many of the inflammatory mechanisms responsible for the pathological changes of PM, DM and IBM are similar to those mediating damage in other inflammatory diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), so it seems likely that genetic factors will similarly be involved in the development and expression of PM, DM and IBM.

Establishing the extent of involvement of these genetic mechanisms in PM, DM and IBM is of great importance, since understanding the aetiological mechanisms of any disease may eventually permit the development of specific and therefore more effective therapies.
Primary Objectives:
To identify and characterise the disease susceptibility genes associated with the development and clinical characteristics of the primary inflammatory muscle diseases, PM, DM and IBM.
Secondary:
To gain further insights into the aetiological mechanisms responsible for the development of the primary muscle diseases PM, DM and IBM and ultimately identify new therapeutic targets for treatment.

64. Full Title: Study of clinical and radiological changes in teenagers with Duchenne muscular dystrophy theoretically treatable with exon 53 skipping (Pre-U7)
Status: Closed to Recruitment
Sponsor: Genethon
Funder: Genethon
London
PI: Prof. Francesco Muntoni
Newcastle
PI: Volker Straub
Patients recruited 5 Target 5
PreU7-53 is a natural history study. The objective is to monitor the clinical and radiological course of upper limb muscle impairment in patients with DMD, potentially treatable with AAV-

mediated exon 53 skipping (i.e.: deletions exons 10-52, 45-52, 46-52, 47-52, 48-52, 49-52, 50-52, 52 of the dystrophin gene), and to assess serum and urine biomarkers to monitor non-invasively disease progression, and finally to assess the prevalence of immunity against adenoviral vectors in this relevant DMD population.

65. SMA registry
PI: Prof. Hanns Lochmuller
Status: Ongoing
The UK SMA (Spinal Muscular Atrophy) registry is for all patients living the UK and Ireland who are affected by all types of Spinal Muscular Atrophy. The aim of the registry is to encourage genetically diagnosed SMA patients to register so that they may be considered for relevant clinical trials, receive the most up to date information regarding standards of care for their disease and help provide the research community with an understanding of disease prevalence. People with SMA, or the parents/guardians of children with SMA, can register themselves online. The UK SMA registry was set up in 2008 as a collaboration between TREAT-NMD and the Jennifer Trust for Spinal Muscular Atrophy, and is part of the TREAT-NMD Global SMA Registry. Since 2012, the registry is supported by the Jennifer Trust.

66. UK Myotonic Dystrophy patient registry
PI: Prof. Hanns Lochmuller
Status: Ongoing
The UK Myotonic Dystrophy Patient Registry is an online patient driven resource launched in May 2012. The primary aim of the registry is to facilitate and accelerate the planning, design and recruitment of clinical research while also providing a snapshot of the myotonic dystrophy population in the UK. The registry collects an internationally agreed dataset with the majority of information provided by the patient themselves, additional clinical and genetic details are provided by their neuromuscular specialist. The registry is funded by the Myotonic Dystrophy Support Group and Muscular Dystrophy Campaign with support from the TREAT-NMD Alliance.

67. Global FKRP registry
PI: Prof. Volker Straub
Status: Ongoing
The Global FKRP Registry is an international registry for all persons affected by conditions caused by a mutation in the *Fukutin-Related Protein* (*FKRP*) gene, namely Limb Girdle Muscular Dystrophy type 2I (LGMD2I), and also the rarer conditions Congenital Muscular Dystrophy type 1C (MDC1C), Muscle Eye Brain Disease and Walker-Warburg Syndrome. The Registry aims to facilitate recruitment into clinical trials by identifying patients more readily, accelerate research, and provide more detailed knowledge about the natural history and prevalence of FKRP-related muscular dystrophies, whilst keeping patients informed. The Registry was set-up in 2011 as an online patient driven registry and is currently supported by the LGMD2I Research Fund.

68. GNE myopathy-Disease Monitoring Programme (GNE-DMP): A registry and prospective observational natural history study to assess HIBM disease
PI: Prof. Hanns Lochmuller
Status: Ongoing
The Disease Monitoring Program is a public-private partnership between Ultragenyx Pharmaceutical Inc. (USA) and Newcastle University (UK). The program was designed to collect data on clinical presentation and progression of GNE myopathy to improve knowledge and support treatment development. The unique structure of the program allows a combination of longitudinal data collected through an online global patient registry and a hospital based natural history study in a single platform. The Natural History study is performed by selected centres in Europe, Middle East and North America. Anonymous data gathered through the registry will be accessible to the medical and research community, patients, families and patient organisations

upon approval from the Steering Committee and Ethical Committee in the hope that this information will provide insight into the disease, and help drive clinical trials and research that could lead to better treatment strategies.

69. SMA REACH UK
Spinal Muscular Atrophy Research and Clinical Hub UK
Status: Open to recruitment
Funder: UK SMA charity: SMA TRUST
Sponsor: Great Ormond Street Hospital
London
CI: Prof. Francesco Muntoni
Newcastle
PI: Prof. Katie Bushby
Target: ~70 Recruitment: 52
The primary aim of this project is to establish the first national clinical and research network named SMA REACH UK (SMA Research and Clinical Hub UK) to establish a national agreement on clinical and physiotherapy assessment and standards of care. We propose designing, piloting and expanding an electronic database created to streamline the collection of data for patients with SMA. This UK SMA database would be a unique infrastructure started at GOSH and Newcastle which would soon be built up and accessible to specialist centres across the UK who treat patients with SMA.

70. OPTIMISTIC
Observational Prolonged Trial in Myotonic Dystrophy type 1 to Improve Quality of Life Standards, a Target Identification Collaboration
Status: Open
Funder: EU Seventh Framework Programme
Sponsor: The Newcastle upon Tyne Hospitals NHS Foundation Trust
Newcastle
CI/PI: Dr Grainne Gorman
UK Target 72; recruited 37
OPTIMISTIC is a two-arm, multi-centre, randomised controlled trial designed to compare a tailored behavioural change intervention against standard patient management regimes. It is expected that the trial and outcome work will lead to new clinical guidelines for DM1 management. The intervention comprises cognitive behavioural therapy (CBT) and graded physical activity, both of which aim to achieve a more active lifestyle. The effectiveness of this intervention, together with any adverse events associated with it, will be compared to standard patient management. Outcome measures will be measured at baseline, 5 months, 10 months (the end of the intervention period) and at 6-months post intervention (i.e. 16 months from baseline).

71. FSHD NH Study
A multicentre collaborative study on the clinical features, expression profiling, and quality of life of infantile onset facioscapulohumeral muscular dystrophy
Status: Closed to recruitment
Sponsor: CINRG
CI: Prof. Kate Bushby
PI: Dr Michel Guglieri
This multicenter study will be conducted at participating US and International CINRG sites. Fifty individuals with infantile onset (diagnosed at <11 years of age) and genetically confirmed FSHD will be recruited for a cross-sectional study of pediatric FSHD. This will include children and youth (less than 18 years old) with FSHD who are currently followed in pediatric neuromuscular centers, as well as adults (18 years or older) with FSHD who are identified as having infantile onset of disease by chart review, clinical exam, and genetic confirmation. Our goal is to have close to 25 individuals with early infantile onset (<5 years) and 25 with late infantile onset (5 to 10 years) of FSHD in order to compare their clinical phenotypes and health-related outcomes.

72. Genotype-Phenotype in inherited neurodegenerative diseases
Status: Open
Funder: Wellcome Trust
Sponsor: Newcastle upon Tyne NHS Hospitals Foundation Trust
PI: Prof. Patrick Chinnery
The principle aim of this project is to define the genetic basis of undiagnosed inherited neurodegenerative disorders.
The secondary aim is to define the extent of the clinical phenotype of these novel neurodegenerative disorders.
Long term aims will be the identification of new disease mechanisms potentially amenable to the development of effective treatments for these neurodegenerative disorders.

73. CBYM338B2302: A Prospective Natural History Study in Sporadic Inclusion Body Myositis (sIBM)
Sponsor: Novartis
Status: Open to recruitment
PI: Dr Pedro Machado/ Prof. Michael Hanna
Recruitment target: 30
This is a longitudinal, multinational, multicenter natural history study of patients diagnosed with sIBM
The study aims to characterize the clinical progression and functional impact of sIBM on patients and their caregivers over time assessed by functional performance measures and patient-reported outcomes (PROs). The association between functional impairment and long-term outcomes, such as loss of mobility and falls, will be examined. In addition, this study will characterize both the economic and humanistic burden of this disease. Furthermore epidemiological data collected in this study will document basic demographic characteristics of the sIBM patient population. The primary objective is to describe patient-reported functional impact of sIBM over time as measured by the Sporadic Inclusion Body Myositis Physical Functioning Assessment (sIFA).

74. Jain Foundation natural history and clinical outcomes study of dysferlinopathy (limb-girdle muscular dystrophy type 2B)
Status: closed to recruitment
Sponsor: The Newcastle upon Tyne Hospitals NHS Foundation Trust
Funder: Jain Foundation
Newcastle
CI/PI: Prof. Kate Bushby
Target 20, recruited 43
A clinical outcome study for Dysferlinopathy. To define the natural history of dysferlinopathy in a large unselected patient group with respect to age and nature of onset, progression and presence of complications via existing and expanded registries and databases. Study a selection of possible outcome measures for dysferlinopathy trials over a three year period in a multicentre evaluation of 150 patients based in centres of excellence for muscular dystrophy diagnosis and management. Extend the existing registry activities co-ordinated by the Jain Foundation to ensure a comprehensive.

75. Full Title: Mito Exome Sequencing Study
Status: Open
Sponsor: Guys and St Thomas
Funder: Lily Foundation
Newcastle
PI: Dr Robert McFarland
London
CI: Charulata Desphande
PI: Prof. Michael Hanna

Patient target: 100 families
The primary aim of our study is to harness recent advances in genetic testing to try and identify nuclear genes causing mitochondrial disorders in young children. We will use Next Generation Sequencing technology to test children with confirmed mitochondrial disorders in whom routine testing has failed to provide a genetic diagnosis/explanation. Gene identification will help us to develop more comprehensive tests for the diagnosis of mitochondrial disorders. It will also allow us to manage the genetic risk within a family. We would also like to interrogate the data to identify interacting proteins that might inform treatment of these conditions.
For more information please contact Julia Maddison at julia.maddison@ncl.ac.uk

76. Full Title: Reproductive Decision Making in Mitochondrial Disease
Status: Open
Sponsor: Newcastle Upon Tyne NHS Foundation Trust
Funder: MRC
Newcastle
PI: Prof. Doug Turnbull
Target 30, recruited 6
With newly available and emerging reproductive techniques, mitochondrial patients now have even more reproductive options than ever before to consider. There is currently no published research exploring how mitochondrial patients make these reproductive decisions, comparing the past and present decision making, how strongly patients feel for or against their current and possible available options and how patients would like this complex information presented to them. Primary Objectives is to investigate women with mitochondrial disease experiences of reproductive decision-making.
Specifically this study will explore:

- To explore women's experiences of living with a diagnosis of Mitochondrial Disease
- To explore women's knowledge about risk of transmission to their children, genetic testing and reproductive techniques.
- To explore the impact of health professionals, family and other information sources on decision-making
- To explore women's information needs

For more information please contact Julia Maddison at julia.maddison@ncl.ac.uk

77. Full Title: Incidence of complications of pregnancy in patients diagnosed with mitochondrial disease or carrying a mitochondrial DNA mutation
Status: Closed to recruitment
Sponsor: Newcastle Upon Tyne NHS Foundation Trust
Funder: MRC
PI: Dr Robert McFarland
Target: 200 (UK) Recruited 151 (80 patients, 71 controls)
Approximately 1 in 3,500 women are affected by a mutation causing mitochondrial disease, many of whom are of childbearing age. However there is currently no information available for these women regarding the risk of complications in pregnancy. In patients who already have impaired mitochondrial function, it might be predicted that the increased respiratory demand during pregnancy and particularly at the onset of labour may lead to the development of serious complications. This study aims to look at whether increased energy demand can lead to complications of pregnancy in the female population of patients diagnosed with Mitochondrial Disease or carrying a mitochondrial mutation. Assessment will be undertaken at a face to face interview using a structured questionnaire or via a postal questionnaire with telephone assistance at a predetermined time convenient to the participant

For more information please contact Julia Maddison at julia.maddison@ncl.ac.uk

Closed Studies

78. NON-DYSTROPHIC MYOTONIAS: GENOTYPE AND PHENOTYPE CORRELATION AND LONGITUDINAL STUDIES
Status: Closed
Sponsor: University College London
Funder: National Institutes of Health (NIH – USA)
PI: Prof. Michael Hanna
Patients recruited: 20
This multi-centre project involves a prospective, cross-sectional and longitudinal natural history in non-dystrophic myotonias (NDM).
The aim is to collect standardized data from NDM patients, to include clinical symptoms, exam findings, as well as the results of strength, functional, and electrophysiological testing. Genetic testing will permit precise identification of individual NDM subtype. This information will allow for the identification and implementation of appropriate endpoints in studies of potential treatments.
This is a NIH funded study. Twenty patients were enrolled at the National Hospital for Neurology and Neurosurgery.
Brain. 2013 Jul; 136 (Pt 7):2189-200. DOI: 10.1093/brain/awt133. Epub 2013 Jun
PMID: 23771340
[PubMed - in process]

79. ANDERSEN-TAWIL SYNDROME: GENOTYPE AND PHENOTYPE CORRELATION AND LONGITUDINAL STUDY
Status: Closed
Sponsor: University College London
Funder: National Institutes of Health (NIH – USA)
PI: Prof. Michael Hanna
Patients recruited: 11 target >10
Andersen-Tawil syndrome is a neuromuscular disorder caused by a mutation in the KCNJ2 gene which codes for the inwardly rectifying potassium channel Kir2.1. A number of different mutations in this gene have already been identified in affected individuals. This disorder is characterised by the triad of periodic paralysis, developmental abnormalities and cardiac arrhythmias.
This project is a natural history trial into Andersen-Tawil Syndrome. The aim of the trial is to study the relationship between the genetic abnormalities underlying the disorder and the diverse clinical features.
Eleven patients have been enrolled so far at the National Hospital for Neurology and Neurosurgery.

80. EPISODIC ATAXIA SYNDROME: GENOTYPE-PHENOTYPE CORRELATION AND LONGITUDINAL STUDY
Status: Closed
Sponsor: University College London
Funder: National Institutes of Health (NIH – USA)
PI: Prof. Hanna
Patients recruited: 36 target >20
Episodic Ataxia Syndrome is a rare, genetic disease that causes recurrent episodes of dizziness and incoordination.
The majority of cases are likely caused by an inherent genetic mutation. However in some patients the mutation is unidentifiable. The purpose of this study is to collect prospective standardized data from subjects to better define the clinical phenotype of the EAs and to establish clinically relevant endpoints for use in therapeutic trials.
The study will also:
- Fully characterize the clinical spectra and the natural history of genetically defined EA.
- Systematically investigate phenotypic differences between EA subjects harboring KCNA1/CACNA1A mutations and those that do not.

This proposal involves a multi-center cross-sectional data collection analysis as well as a prospective longitudinal study. Since EA is a chronic disease whose course is measured in years rather than months, the subjects will be followed longitudinally at a yearly interval for a period of two years. *Brain 2014: 137; 1009-1018*

81. OUTCOME MEASURES IN SMA TYPE II AND III
Status: Complete
Sponsor: UCL Institute of Child Health
Funder: SMA Europe
London
PI: Prof. Francesco Muntoni
Newcastle
PI: Prof. Kate Bushby
Recruitment target (UK) 23; Patients recruited: 26
Description: The primary aim of this project is to establish, for the first time, a clinical network involving most of the leading neuromuscular centres in Europe and to enable them to have common outcome measures in order to be ready for forthcoming multi-centre trials on SMA type II and III
Objective(s)
Primary objective:
• To establish a clinical network involving most of the leading neuromuscular centres in Europe enabling them to have common outcome measures on SMA type II and III.
Secondary objectives:
• To ensure the functional scales used are suitable and clinically relevant for future trials, that we understand how the different measures relate to one another and how they may change over a 12 month period
This prospective longitudinal natural history study will be performed in two cohorts of patients with SMA type II and III identified according to their level of functional motor ability (ambulant/non ambulant). Inclusion criteria and methods will be different in the two cohorts and will be described separately. We have considerable retrospective data on SMA but very little planned data and none using the range of outcome measures proposed.
Further information can be obtained from the Trials Coordinator or Research Physiotherapist on 020 7905 2639.

82. PERIPHERAL NEUROPATHY OUTCOME MEASURES STANDARDISATION STUDY (PERINOMS)
Status: Complete
Sponsor: Erasmus Medical Center
PI: Dr Michael Lunn
Patients recruited: 110; overall target 120
The current study aims to expand the clinimetric knowledge on outcome measures at various levels of outcome (pathology, impairment, activity & participation limitation, and quality of life) in autoimmune polyneuropathies, particularly in GBS, CIDP, MMN, MGUSP, and autoimmune small fibre neuropathies (AI-SFN). Also, the general applicability of an autonomic symptoms scale plus some selected activity limitation scales will be examined.
Outcome measures will be assessed in a cross-sectional and longitudinal group of patients at the level of:
- Pathology: Intraepidermal nerve fibre (IENF) density will be assessed in patients with GBS, CIDP, MGUSP, and AI-SFN (in sarcoidosis). IENF density will be examined regarding its correlation with other outcome measures (validity), its reliability (intra-observer and inter-observer), and its responsiveness to clinical changes over time.
- Impairment: comparison studies, evaluating the validity, reliability, and responsiveness will be performed between MRC sumscore versus NIS motor subset, INCAT sensory sumscore versus

NIS sensory sumscore, and hand-held Vigorimeter versus Jamar dynamometer. Also, the correlation of electrophysiological studies with other impairment outcome measures will be evaluated. Finally, the scientific soundness of the modified Dutch composite autonomic symptoms scale (mdCompass) will be examined.
- Activity limitation: comparison studies, evaluating the validity, reliability, and responsiveness will be performed between the ODSS and an overall neuropathy limitations scale (ONLS). Also, a newly devised weighted (based on Rasch analyses) activity and participation scale will be constructed, aiming specifically on the limitations in patients with polyneuropathy.
- Quality of life: Disease-specific versus generic quality of life measures will be assessed, determining their clinimetric soundness and by comparison studies in the various polyneuropathy groups. The ultimate goal of the current study will be the presentation of a *specific* minimum core set of outcome measures to be used in future clinical and follow-up studies in patients with polyneuropathy, mainly those patients with autoimmune mediated polyneuropathies. The study will be performed in collaboration with several local, European, and USA neurological centres with great experience in dealing with inflammatory neurological disorders.

83. A Study of Biological Prognostic Factors for IGM Paraproteinemic Anti-Mag Associated Peripheral Neuropathy
Sponsor: UCL
Status: Closed
PI: Dr Michael Lunn
Recruitment target: 45 patients
Anti-MAG neuropathies have a variable severity and some have a non-significant response to immunotherapies, but all have significant risks of potentially severe adverse effects from treatment. It seems important to find predictive factors in order to determine which patients have a high risk of evolution to severe disability so treatment would be targeted to appropriate patients. We suggest studying factors which could influence the disease evolution including molecules that regulate the monoclonal IgN secreting B-cells (BAFF, APRIL, inflammatory cytokines), molecules that may modulate the alteration of the blood-nerve barrier (inflammatory cytokines, VEGFs, angiopoietins).

This is a retrospective cohort study, including patients from the National Hospital for Neurology, London, UK, and from the University Hospital of Rennes, France.

The objective is to determine biological factors in blood and CSF that could be predictive of severity of neuropathies associated with IgM anti-MAG antibodies.

Exercise Studies

Open Trials

84. Aerobic training in Charcot-Marie-Tooth disease and Inclusion Body Myositis.
Status: Recruiting
Sponsor: University College Hospitals
PI: Dr Gita Ramdharry
The specific objective of the present study is to investigate the effect of aerobic training in two common neuromuscular diseases (NMD): Charcot-Marie-Tooth disease (CMT) and Inclusion Body Myositis (IBM). These diseases result in progressive muscle wasting and substantial morbidity and disability. The effect of aerobic training on fitness levels, muscle strength and function will be systematically examined. This study will also monitor the safety, feasibility and impact on quality of life of this type of exercise training in these groups.
Sixty subjects, (30 from each disease group, aged between 18 and 75), will be recruited from the neuromuscular clinics at Queen Square. Both disease groups will be investigated concurrently with

the same methods but will be viewed and analysed as separate studies. A crossover design will be used with training and control periods. The trial will span three years with each subject participating for a 34 week period. For the training intervention, participants will train in select local gyms and train on a bicycle ergometer.
The primary outcome measure for this study is maximum aerobic capacity during exercise testing. There will also be measures of muscle strength, body composition, and activity levels. In addition the study will investigate non-motoric effects of exercise such as mood, motivation, sleep and fatigue.
For further information please contact Dr Amanda Wallace, Amanda.wallace@uclh.nhs.uk

85. Full Title: Exploring the causes of falls and balance impairments in people with neuromuscular diseases
Status: Recruiting
Sponsor: University College Hospitals
Funder: NIHR
PI: Dr Gita Ramdharry
Falls are commonly reported by people with neuromuscular disorders but to date there has been little formal investigation of this problem. Frequent falling increases the risk of injury and reduces mobility due to avoidance of activities perceived to increase the threat of falls. The aim of this study is to ascertain falls risk from measurement of falls incidents, balance impairment and clinical presentation in people with different types of Charcot-Marie-Tooth (CMT), Distal Myopathy (DM) and Sensory Neuropathy (SN) with healthy controls. Measurements of static, anticipatory and reactive balance impairment and prospective falls events will be used to ascertain relationships with clinical presentation in people with different types of CMT, DM and SN. The three pathologies have been chosen for comparison as this will allow some discernment between the sensory and motor contributions to falls. *Physiotherapy. 2014 mar; 100(1):61-5. doi:10.16/j.physio.2013.06.002. Epub 2013 Aug 15. PubMed PMID: 2395402*

86. PHYSICAL ACTIVITY AND INCLUSION BODY MYOSITIS
Status: Recruiting
Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust
Funder: MRC
PI: Dr Michael Trenell
Collaborating site MRC Centre London
Recruitment: 500 recruits expected, across 5 disease sites
The aim of this study is to collect data on day to day physical activity levels and metabolic control in individuals with chronic disease.
DESIGN:
Participants will be identified from chronic disease clinics by the following lead clinicians: Stroke-Prof. Gary Ford, Neuromuscular disorders-Prof. Kate Bushby, Metabolic disorders-Prof. Roy Taylor, fatigue-Prof. Julia Newton and Ageing-Prof. Julia Newton. An equal sample of male and female participants will be used in the study which will be up to 100 patients in each disease group.
METHODOLOGY:
Step 1: Relevant practitioners will highlight possible candidates for the study.
Step 2: Visit 1: At the start of the study participants will either be asked to attend Newcastle University's Campus for Ageing and Vitality (Newcastle General Hospital), or if they are an inpatient will be visited on the ward. Participants will be provided with an information sheet about the study. They will be given the opportunity to talk with the team and ask questions. Once fully informed, participants will provide signed informed consent.
Participants will be asked to fill in a disease screening questionnaire at the start of the process. The height and weight of the participants will be recorded and this information will be entered into the physical activity monitors. Instructions will be provided as to how to use the monitors. A

resting blood sample may also be taken at this point. This will be analysed for glucose, insulin, lipid profile and liver function.
Step 3: Participants will wear the arm monitors for five days including one weekend day. Step 4: Visit 2: At the end of the five day period participants will attend the research centre again or attend a pre-arranged session either at their home work place or on the ward to return the activity monitor. Here they will complete a brief physical activity questionnaire and two brief fatigue questionnaires. Data from the physical activity monitor will be fed into a computer. Each participant will be provided with a printout of their weekly activity levels and given the opportunity to discuss their results.
For information about recruitment contact Julia.maddsion@newcastle.ac.uk.

87. EXERCISE AND SARCOPENIA
Status: Recruiting
Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust Funder: MRC
PI: Dr Grainne Gormann
Collaborating site MRC Centre London (Recruitment at Newcastle only)
Target: 36
Recruited: 33

Sarcopenia, which is a complex multifactor process, has significant implications on quality of life, performance of daily activities, maintenance of independence and on projected healthcare costs. Studies show that low physical activity correlates with poor mitochondrial function. Conversely, exercise correlates with better mitochondrial function, clinical improvement and improved perceived quality of life. Endurance training has been proven to be safe and efficacious in mitochondrial disease which may provide a model for the aging process albeit in an accelerated form with biochemical, histological and genetic changes seen in aged muscle also found in various mitochondrial conditions.
Aims:
1. To assess the rate and extent of motor unit loss in the eighth decade of life- cross-sectional (time 0) and longitudinal analysis (end of study)
2. To correlate the extent of motor unit loss with histological correlates and the development of sarcopenia
3. To assess the impact of exercise on the rate and extent of motor neuron loss
4. To observe whether endurance training initiated in late middle age prevents loss of muscle strength and mass in senescence
5. To assess the impact of neuronal loss on the inability to retain gains made in muscle strength following training after the 7th decade of life
6. To characterise effects of exercise upon neural activity, muscle oxidative capacity and mitochondrial and satellite cell plasticity with age.
Method: Thirty six (36) female participants, matched for body mass index who do not take regular exercise will be invited to participate: years 30-40, 50-60 and 70+ . Inclusion criteria will be capacity to undertake cycling exercise and ability to give informed consent. Exclusion criteria will be co-existing active coronary artery disease or steroid therapy.
These patients will be recruited via the media and social support groups. All expenses (travel, accommodation and meals) will be paid for from the research grant.
The study will take place over 16 weeks. Participants will attend the study centre for 7 visits in total. The study will include 2 main visits at the beginning and end of the study. Each main visit will last 3 days. There will also be 5 one day visits.
For information about recruitment contact Julia.maddison@newcastle.ac.uk

88. Exercise, cognition and brain vitality
Status: Open

Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust
Funder: MRC
Newcastle
PI: Dr Grainne Gorman
Patients recruited: 28
Research is emerging suggesting the positive effect of aerobic exercise on many areas of cognition, such as visuospatial processing, memory, attention, reaction time, and executive function, with the most marked improvements in executive functioning. These improvements have been found for both healthy and cognitively impaired individuals. As such, exercise may prove a useful intervention for many patients with age related illnesses. The study will investigate the effects of exercise on cognitive functioning using a battery of tests to assess a range of key cognitive functions. Performance on cognitive tests will be examined before exercise intervention, immediately following completion of the intervention to examine potential changes in cognition. Cognitive testing will be repeated six months post exercise intervention to determine whether cognitive changes have been maintained.

Closed Trials

89. STRENGTHENING HIP MUSCLES TO IMPROVE WALKING DISTANCE IN PEOPLE WITH CHARCOT- MARIE-TOOTH DISEASE
Status: Completed
Sponsor: University College London Hospitals
Funder: Muscular Dystrophy Campaign (MDC)
PI: Prof. Mary M. Reilly
Patients recruited: 32 target: 32
Charcot-Marie-Tooth (CMT) disease is a form of hereditary peripheral neuropathy.
People with CMT present with weakness, wasting and sensory loss as a result of degeneration of the long peripheral nerves supplying the distal muscles.
The aim of this study will be to investigate the efficacy of a 16 week home based programme of training to increase hip flexor muscle strength and walking endurance. Additional measures of gait speed, exertion, fatigue, disability and general activity will also be recorded. Baseline impairment measures will be obtained to ascertain predictors of strength gains.

This study will use a single blinded, randomised cross over design to investigate if training the hip flexor muscles will strengthen the hip flexor muscle and improve walking endurance in people with all types of CMT.
The trial will include people aged between 18 and 70 years, who have been diagnosed with CMT on the basis of genetic tests (where possible), family history and neurophysiology testing. Each subject will be involved with the study for a 40 week period.
Results published Journal of the Peripheral Nervous System, 2011; 16(S3):S115

90. EXERCISE TRAINING IN PATIENTS WITH MITOCHONDRIAL DISEASE: ASSESSING THE BENEFITS
Status: Closed
Sponsor: University Newcastle
Funder: Muscular Dystrophy Campaign (MDC)
PI: Prof. Doug Turnbull
Collaboration site MRC Centre London (Hanna)
Patients recruited: 9 Newcastle; 0 London
Mitochondrial myopathies are a very important group of muscle diseases associated with weakness, pain and fatigue. At present, treatment options are very limited.
Exercise therapy has been found to have some benefit in this group of patients and we wish to explore this further in terms of both strength and endurance.

The aim of this study is to demonstrate that strength exercise training is an effective approach to therapy in certain patients with mitochondrial myopathy, specifically those with sporadic mutations in mitochondrial DNA. Based on our previous research studies, we believe that such training will improve muscle strength, mitochondrial function, exercise tolerance and overall quality of life.
The main objectives are:
To confirm that endurance training in patients with mitochondrial abnormalities improves quality of life, exercise tolerance and oxidative capacity.
To determine the ability of resistance muscle strength training to improve skeletal muscle strength and oxidative capacity by incorporation of satellite cells into mature myofibres.
Participants are expected to commit to an exercise training and testing over a period of 4 to 8 months.
The study will include patients between the ages of 18 and 65 years who have had a previous muscle biopsy showing a defect in skeletal muscle mitochondrial DNA that is either in the form of a sporadic point mutation or single large-scale deletion. Patients who have this type of mutation and do not have any family members that are affected and have no major cardiac involvement, hypertension, pulmonary or peripheral vascular disease that may complicate findings.

91. CARDIAC ADAPTATIONS TO EXERCISE IN MITOCHONDRIAL DISEASE
Status: Closed
Sponsor: Newcastle upon Tyne Hospitals NHS Foundation Trust
Funder: MRC
PI: Prof. D M Turnbull/Dr MI Trenell,
Patients recruited: 39
Twenty four people with mitochondrial disease will take part in the study. Participants will undergo cardiac, cognitive and movement examination and then they will be randomised into two groups. They will receive either; exercise counselling and support (n = 12) or continue standard care (n = 12) over a 16 week period. At the end of the 16 week period baseline measures will be repeated. Participants to be studied will have biopsy proven mitochondrial disease (age 18−60 years; BMI 20−35 kg/m2; and do not take part in regular exercise). Subjects with heart disease that would produce an adverse response to exercise will be excluded. Subjects with significant kidney disease or in vivo ferrous material will be excluded also as these are contra-indications to the use of gadolinium-based contrast agents and magnetic resonance imaging respectively. Magnetic resonance and echocardiographic evaluation of cardiac function as well as movement and cognitive function will be assessed at baseline and at 16 weeks. A progressive exercise test will be undertaken at baseline to establish maximal aerobic capacity and evaluate for an adverse response to exercise.
The patient exercise group will be matched with a control group of individuals without known mitochondrial disease who will undergo the same evaluation and training regime (n = 12)..
In total, the study will require each participant to attend the research facility for three visits for metabolic examination. The exercise groups will be requested to attend 48 exercise sessions over 16 weeks.
For information about recruitment contact Julia.maddison@newcastle.ac.uk.

Imaging Studies

Set-up Phase

92. A study using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) in patients with Limb girdle muscular dystrophy 2I ; an assessment of skeletal and cardiac muscle damage
Sponsor: Newcastle upon Tyne NHS Hospitals Foundation Trust

CI: Prof. Volker Straub
PI: Prof. Volker Straub
We intend to rescan and assess the original 2009-2010 cohort of LGMD 2I patients with both functional and imaging measures. The overall aim is to evaluate the non-invasive measurements of fat fraction to quantify muscle pathology. We aim to evaluate the rate of change of muscle fat replacement over the five year period in individuals with LGMD2I. This shall be achieved by measuring morphology by MRI and comparing the results to the original study from 5 years previously. We will on the same occasion assess cardiac function parameters using MRI using (i) cine imaging to evaluate systolic and diastolic function, (ii) cardiac tagging to measure wall motion and torsion and (ii) late gadolinium enhancementand extracellular volume measurement to assess myocardial fibrosis.

Open Trials

93. Magnetic Resonance Imaging Characteristics of Inflammatory Neuropathies – a pilot study
Status: Open to recruitment
Sponsor: University College London Hospitals
PI: Dr Michael Lunn
Patients recruited: 20: 10 patient; 10 controls
The assessment of patients with peripheral nervous system (PNS) disease is currently mainly dependent on clinical examination, neurophysiological tests and occasionally nerve biopsy. Clarification of nerve imaging characteristics in chronic inflammatory demyelinating polyneuropathy (CIDP) could alleviate the need for invasive procedures such as nerve biopsy in cases where there is uncertainty in the clinical diagnosis.
Magnetic resonance imaging (MRI) has been widely applied to neurological diseases of the central nervous system, but to a much lesser extent diseases of the PNS. Research in inflammatory neuropathies has included traditional T1 and T2-weighted sequences; some more recent work in mainly focal entrapment neuropathies has looked at novel MRI sequences such as diffusion tensor imaging.
CIDP is an immune mediated condition characterised by progressive or relapsing motor and sensory deficits in all four limbs. It is a treatable condition and often responds to immunomodulatory treatment. Currently the diagnosis is based on a combination of clinical, neurophysiological and supportive criteria. Diagnosis can be difficult as the causative pathology is often proximally sited in the nerves, and their proximal portions are less anatomically accessible to neurophysiological examination.
Recent work in our unit has demonstrated that the sciatic nerve area in CIDP patients is significantly enlarged compared with controls, but with substantial overlap between the ranges of values obtained for disease and control groups. Since much of the pathology in CIDP is located at the nerve roots it is important to assess whether enlargement of the roots is able to differentiate between CIDP and controls.
There is no published research documenting the use of novel MRI techniques in patients with CIDP. Diffusion sequences and assessment of the magnetisation transfer ratio (MTR) of nerves may reveal diagnostic characteristics in diseased tissue, as is seen in the brain. Aims:
We aim to clarify the use of MRI for the diagnosis of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMNCB). Using 3T MRI, we will use both conventional and novel quantitative MRI sequences to examine the nerve roots, plexuses, sciatic nerves and forearm nerves of 10 patients each with CIDP, MMNCB and 20 healthy volunteers. We will quantify nerve root cross sectional area in cervical and lumbar regions in patients with CIDP, MMN and healthy controls. We will explore imaging characteristics of the sciatic nerve in patients with CIDP versus healthy controls. We will define imaging characteristics at sites of conduction block in nerves of patients with MMNCB. In a separate group of patients with suspected inflammatory neuropathy we will compare MRI to pathological findings on nerve biopsy. MRI may be shown to be a useful non-invasive diagnostic tool.

For further information, contact Dr Jasper Morrow, j.morrow@ucl.ac.uk

94. 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: Closed to Recruitment
Sponsor: University College London Hospitals
Funder: MRC
PI: Prof. Tarek Yousry/Dr John Thornton
Patients recruited: 72: 40 patients; 32 controls
Magnetic resonance imaging (MRI) is a key tool in the diagnosis and management of a number of diseases. Despite the wide use of MRI in several clinical settings, so far its role in neuromuscular disease has not been well established. The current standard for the diagnosis of neuromuscular disorders includes clinical examination, electrophysiological investigations, biopsy and genetic testing. Due to the nature of the involvement of prominent muscles and peripheral nerves in these disorders it is proposed that MRI could play a prominent role in understanding of neuromuscular disease.
This study aims to investigate the use of MRI as a tool in the study of nerve and muscle diseases by focusing on two particular neuromuscular diseases, one primarily neuropathic and one principally myopathic. Two separate patient cohorts with neuromuscular disease will be recruited. Forty patients with Sporadic Inclusion Body Myositis (IBM) and 40 patients with genetically confirmed Charcot Marie Tooth Disease (CMT). In addition to the two patient cohorts, two groups of healthy volunteers each of size 40 will act as comparators for the disease groups. Each of the patients enrolled in the study will undergo an MRI scanning session in which the quantitative MR techniques developed in Phase 1 with the health volunteers will be applied. In addition to the MRI scanning sessions, each patient will undergo a clinical examination to record the main clinical features of their disease status including an electrophysiological nerve conduction assessment. In the final phase of the study, a sub-group of the patients will then be followed-up at 6 month intervals for 5 years in a longitudinal natural history study of IBM and CMT that focuses on the MR methods and clinical findings that were shown to be most illuminating.
Changes over time in the MRI parameters in the diseased groups and Healthy volunteers will be compared.
Objectives:
To detect, using quantitative magnetic resonance imaging (qMRI), the changes in the nerves and muscles of patients with inclusion body myositis or Charcot Marie Tooth disease, and to relate these changes to the measurable clinical and neurophysiological features in these diseases. This will allow the value of various qMRI techniques as markers of disease activity and progression to be tested.
Secondary objectives of the study include:
The development of novel quantitative MR techniques for targeted assessment of the human neuromuscular system.
To more fully characterise both the magnetic resonance imaging and clinical features of inclusion body myositis or Charcot Marie Tooth disease as compared with healthy individuals and to study the progression of these characteristics with time over a period of 5 years.
For more information about the study please contact Dr Jasper Morrow at j.morrow@ion.ucl.ac.uk.
European Radiology 2014 April 20; Under review with Lancet Neurology

95. Magnetic Resonance Imaging as an outcome measure in Motor Neuropathies: a pilot study
Status: Recruiting shortly

Sponsor: UCL
PI: Prof. Michael Hanna
The development of novel therapies for motor neuropathies necessitates the search for a reproducible outcome measure which can sensitively monitor disease progression. Muscle magnetic resonance imaging (MRI) is an excellent candidate due to its reproducibility and observer independence. We plan to investigate various parameters obtained through muscular MRI as longitudinal biomarkers in diseases of the motor neuron with different speeds of disease progression: amyotrophic lateral sclerosis (ALS), Kennedy's disease (KD) and distal hereditary motor neuropathy (dHMN). Using 3T MRI the research team will perform lower limb imaging with quantitative 3-point Dixon, magnetisation transfer and IDEAL-CPMG sequences in addition to standard qualitative T1 and STIR sequences in 12 patients each with ALS, KD and dHMN as well as 12 healthy volunteers. Detailed clinical data will be collected, including isokinetic and isometric lower limb strength. These assessments will be repeated at a 3 and 12 month interval in ALS patients and at a 6 and 12 month interval in dHMN and KD patients. We will analyse the value of quantitative MRI as an outcome measure in these conditions by analysing both correlation with clinical measures and sensitivity to change over time. Data from this study will be able to be used to establish sample size in clinical trials to evaluate novel therapeutic strategies in these diseases.
MRI has been widely applied to neurological diseases of the central nervous system, but to a much lesser extent diseases of the peripheral nervous system (PNS), and even less frequently to the diseases in this study.
The hypothesis is that MRI can detect changes in the muscles in patients with ALS, KD and dHMN.
The proposed project will take place in two phases, an initial cross-sectional case control study of all patients and volunteers followed by a longitudinal natural history study.
MRI imaging will be performed of thigh and calf muscles at 3 Tesla in a scanning session lasting approximately an hour. All participants will undergo standard MRI imaging with T1-weighted and STIR sequences. The following quantitative MRI techniques will be used: magnetization transfer imaging, T2 relaxometry with IDEAL-CPMG and 3-point Dixon fat quantification. We will not be using gadolinium contrast in this pilot study.
For further information contact Dr Pietro Fratta, Pietro.fratta@uclac.uk

96. A study of Qualitative Magnetic Resonance Imaging in Channelopathies
Status: Open
Sponsor: UCL
PI: Prof. Michael Hanna
The skeletal muscle channelopathies are a heterogenous group of diseases caused by mutations in voltage-gated skeletal muscle ion channels. Broadly speaking, they can be divided into the non-dystrophic myotonias (NDM) and the periodic paralyses (PP).The objective of this retrospective study is to define the presence, frequency and pattern of MRI abnormalities in the lower limbs of patients with genetically proven PP compared with healthy volunteers. Furthermore, we will describe differences in MRI abnormalities in the subsets of PP.
It will involve approximately 40 patients with genetically confirmed periodic paralysis. To allow blinded analysis, in addition to the MRI scans available from 12 healthy volunteers involved in a previous research study, 12 healthy volunteers will undergo standard clinical lower limb MRI.
For more information about the study please contact Dr Matthew Evans at matthew.evans@ucl.ac.uk

Closed Studies

97. MRI IN FKRP-RELATED LGMD2I
Full-Title: A study using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) in Patients with Limb Girdle Muscular Dystrophy 2I; an assessment of muscle damage
Status: Closed
Sponsor: Newcastle NHS Trust
Funder: MRC

PI: Prof. Volker Straub

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

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

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

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

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

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

For further information about the study please contact Dr. Jasper Morrow at j.morrow@ion.ucl.ac.u.k.

Morrow JM *et al. Quantitative magnetic resonance imaging in limb-girdle muscular dystrophy 2I: a multinational cross-sectional study. PLoS One. 2014; 9 (2):e90377.*

Morrow JM *et al. Quantitative Muscle MRI as an Assessment Tool for Monitoring Disease Progression in LGMD2I: A Multicentre Longitudinal Study. PLoS One. 2013;8 (8):e70993.*

98. A Study of Quantitative Magnetic Resonance Imaging to Monitor Disease Activity in Hypokalaemic Periodic Paralysis.

Status: Completed

Sponsor: UCL
Funder: MRC
PI: Prof. Michael Hanna
Recruitment: 24: 12 patients; 12 controls

The commonest muscle channelopathy is hypokalaemic periodic paralysis caused by mutations in the voltage sensor regions of either the muscle sodium channel SCN4A or the muscle calcium channel CACN1AS. From childhood, patients experience disabling episodes of complete muscle paralysis lasting hours to days. In the early years patients recover in between attacks but over time they develop a permanent fixed muscle weakness (myopathy) and often become wheelchair bound. Although there are established treatment strategies which we and other centres in USA and Europe employ and which can reduce attack frequency, we do not have sensitive methods to monitor disease activity or to determine if the treatment regime is fully effective.

Recent data indicate that muscle water content may be a key determinant of muscle function in patients with higher abnormal water content (oedema) correlating with more weakness. Preliminary published data indicates patients with less oedema may have a better prognosis. Furthermore, we currently make decisions to adjust standard treatments based on attack frequency only and this may not be the most reliable way to monitor actual disease activity in affected muscles. In this study we wish to evaluate abnormal muscle water content using MRI applied in the context of the normal current clinical practice and management in this patient group.

In this study we aim to show that patients with hypokalaemic periodic paralysis have abnormal muscle water on MRI which is inversely correlated with muscle strength and sensitive to changes over time. In a wider context than this study, similar techniques may be applied to other muscle diseases, where MRI could guide treatment in clinical practice and act as an outcome measure in clinical trials.

This study has two phases. The first phase is a period of MRI technique refinement in up to 10 healthy volunteers lasting up to two months. The main study phase is a longitudinal case control study and will study a minimum of twelve patients with hypokalaemic periodic paralysis and twelve healthy volunteers who will act as a comparison group for the patients. Assessments will be repeated at a four week interval to see if any changes in clinical parameters are reflected in changes on MRI parameters. One of the inclusion criteria for patient enrolment will be evidence of active disease in order to maximise differences between the two time points.

For further information contact Dr Jasper Morrow: j.morrow@ucl.ac.uk

Data in analysis

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

Status: Closed to recruitment
Sponsor: UCL Institute of Child Health
Funder: GSK
PI: Prof. Francesco Muntoni
Patient target: 15 (UK) Recruited 15 patients, 10 controls

The primary objective of this study is to characterise the differential involvement of muscle groups occurring with disease progression (i.e. as a function of age) using skeletal muscle MRI so as to more precisely define which muscle groups could provide the best markers for therapeutic response in the non-ambulant boys.

The secondary objectives of this study are to

- Measure quantitative imaging changes in DMD muscle over the course of one year using skeletal muscle and dynamic breathing MRI.
- Measure quantitative imaging changes in diaphragm movement occurring with disease progression (i.e. as a function of age) using dynamic diaphragmMRI.

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

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