1. Summary of Mission and Objectives.

Serious muscle wasting neuromuscular diseases (NMD) are individually rare but represent an important unmet health need affecting>150,000 UK children and adults. They cause life-long disability or premature death. ***The Centre's mission is to translate science into experimental medicine and new treatments for children and adults with disabling/fatal neuromuscular diseases.***

The renewed Centre will operate as an MRC-Host University partnership with the following aims:

- Deliver new experimental medicine studies with clinical impact
- Develop and embed six core translational activities to consolidate experimental personalised medicine across the NMD field. These include: stratified cohorts, experimental trials support and coordination, MRI biomarker development, neuromuscular biobank, preclinical models and PhD capacity building training programmes
- Improve access to experimental medicine and new therapies for this neglected patient group
- Add value to major programmes of separately funded (>£60m) Centre PI discovery science

The key disease research themes underpinning the Centre's scientific strategy and major scientific and translational achievements in last four years:

1. *Muscular Dystrophy* (Muntoni, Morgan, Lochmüller, Straub, Morgan, Brown, Wells, Bushby) Delivered ground-breaking experimental medicine proof of principle trials of antisense oligonucleotide therapy in Duchenne muscular dystrophy (DMD) (MRC funded, *The Lancet 2011*)[1]. Highlighted in the MRC Annual Review 10/11 www.mrc.ac.uk/sevenages. Trial design is underpinned by the first rare disease care guidelines to achieve UK NICE accreditation (*Lancet Neurology 2010)*[2]. Four novel genes and novel molecular mechanisms recently discovered.

2. *Neuromuscular Channelopathies* (Hanna, Schorge, Koltzenburg, Bostock, Lochmüller, Palace, Beeson, Manniko, Kullmann) First phase II experimental trial showed clear efficacy of reprofiled mexiletine in genetic myotonia (*Neurology 2011*)[3]. New in vivo clinical electrophysiological tools for channelopathy diagnosis & genetic stratification (*Ann Neurol 2011*)[4]. Genetic evidence supporting a gating pore current as new pathophysiological mechanism identified (*Neurology 09, 11, J Physiol 2010)*[5-7]. New gene (GFPT1) discovered in congenital myasthenic syndrome (*Am J Hum Genet 2011, J. Neurology 2011)*[8,9].

3. *Inherited Neuropathies* (Reilly, Jessen, Hovarth, Koltzenburg, Greensmith, Houlden) First international multicentre phase II experimental trial in commonest genetic neuropathy (CMT1A) (*Lancet Neurology 2010*)[10]. New disease mechanism linked to accumulation of neurotoxic deoxysphingolipids causes sensory neuropathy *(J Biol Chem 2010)*[11]. Discovery of four new genes for inherited neuropathies.

4. *Inclusion Body Myositis* (Hanna, Turnbull, Hilton-Jones, Houlden, Greensmith, Lochmüller) First safety and tolerability study of a non-licensed experimental compound to upregulate heat shock proteins in inclusion body myositis completed. This investigator led study met the primary outcome measure; manipulation of heat shock protein 70 pathway shown to be safe and tolerated in patients with inclusion body myositis; thereby facilitating an efficacy study of this new approach *(Neurology 2012)*[12].

5. *Mitochondrial Diseases* (Turnbull, Hanna, McFarland, Horvath, Duchen, Rahman, Taylor, Chinnery). Resistance and aerobic exercise therapy shown to be safe and effective in mtDNA deletion muscle disease (*Brain 2010)*[13]. Mitochondrial disease mitigated by idebenone therapy in an experimental medicine trial *(Brain 2011)*[14] Mitochondrial DNA disease potentially preventable (*Nature 2010* & MRC Perspectives 10/11 http://perspectives.mrc.ac.uk/chapters/people-populations-and-body-systems)[15].

The original strategy and objectives for the Centre and their delivery over the past four years

We exceeded all agreed objectives; listed here in the same order as in the original application:

- New ***critical mass*** Francesco Muntoni, Jenny Morgan, Hanns Lochmüller, Rita Horvath, Richard Hughes (& entire Cochrane Neuromuscular Unit), Ros Quinlivan & teams all relocated to the Centre.
- We discovered ***new genes, new pathophysiological mechanisms*** and identified potential new targets in NMD *(Nature & Nature Genetics)*[15,16].
- We established the ***national MRC neuromuscular biobank*** with>1800 human cell lines used in >20 science projects and linked to international rare disease biobanks via Eurobiobank.
- We delivered a step change in ***UK natural history/experimental personalised medicine studies*** rising from just 3 to >30 with an increase in patients entered into experimental studies from 29 to >200

(Lancet, Lancet Neurology & Brain)[1,2,10-14]. We lead international experimental medicine initiatives in the NMD field.

- We ***utilise animal models*** in imaging studies, to understand pathophysiology and as ***preclinical models for potential therapeutics for a number of NMD***. These include novel generation antisense for modification of splicing in Duchenne and spinal muscular atrophy mouse models and assessment of hyperglycosylation strategies in dystroglycanopathies. We also use cell cultures from patients with muscular dystrophy and IBM to explore compound libraries for their therapeutic potential.
- We developed a major ***education and training*** translational research programme and trained ten MRC funded PhD students (two clinical, eight non-clinical). We attracted additional PhD students funded by other schemes.
- We established world-leading nationally coordinated ***stratified experimental medicine patient cohorts*** (now>2000 patients) in target NMD: these cohorts are a critical prerequisite for experimental medicine trials/personalised medicine. We provided highly visible, outward looking, collaborative, ***nationally coordinated translational leadership*** including web-seminars, PhD student retreats, workshops and a high profile MRC translational research annual meeting jointly with major UK Muscular Dystrophy Campaign charity attracting >250 scientists (as well as developing new patient partnerships (http://www.cnmd.ac.uk).
- The Centre is an internationally recognised focal point for NMD research with ***national and global collaborations*** (Oxford Neuroscience and Cambridge MRC Mitochondrial Biology Unit, Europe, Japan, USA, Australia). We developed ***industry partnerships*** that delivered new experimental trials and MRI biomarker development (e.g. GSK, Senexis, Prosensa, Shire). We leveraged MRC Centre status to attract additional funding from grant organisations, host institutions & NHS Biomedical Research Centres >£60millon.

None of this progress would have happened without critically important MRC support for the core activities and resulting leverage with host and other funders.

Vision, strategy and objectives for the future

Our ten-year vision is to consolidate the expertise and tools of the combined Centre to enhance experimental medicine in UK NMD. We will deliver new experimental medicine studies in each of the five disease themes. Our continued leadership will change UK NMD clinical practice and embed an experimental trials culture. Like UK cancer care now, UK NMD patients will have the option to enter national stratified cohorts & experimental medicine trials, or will have effective treatments. ***Our strategy*** is to develop the scientific excellence that delivers "know how" and the tools and resources that underpin successful translation. We will build on effective academic, industry, funder and patient partnerships.

Objectives for the Centre over the next five years

1. People to deliver translational research: build on critical mass and recruit two new world-class colleagues: Cossu (stem cell therapy) to London (UCL) and Senderek (neuropathy) to Newcastle (NCL). We will train more students in unrivalled and inspiring educational environments, and prioritise mentoring best young scientists.

2. Maximising added value of core areas to achieve "pull through" from discovery science into experimental medicine: core translational activities will continue to add value to discovery science and we will deliver new experimental medicine and natural history studies in each target disease. We will develop and refine stratified cohorts, biobanks, MRI biomarkers and additional outcome measures as essential platforms for experimental therapy studies. We will refine our use of preclinical cell and animal models to inform study design of novel experimental therapies. We will extend experimental trial culture to more UK clinicians and NMD patients.

3. Advancing neuromuscular gene discovery to identify new therapy targets and new biomarkers Centre PI programmes of next generation DNA sequencing will enable further genetic stratification of cohorts, identify new therapy targets & enhance diagnostics. Biobanked stratified patient material will be key to advance understanding of new gene disease pathophysiology & preclinical therapy development.

4. Antisense strategies to treat NMD: we will target other dystrophin gene exons using different antisense oligonucleotide (ASO) chemistries in collaboration with industry (AVI BioPharma and GSK/Prosensa). We will study a new generation of peptide conjugated antisense oligomers. We aim to develop applications of antisense technology to new NMD such as spinal muscular atrophy.

5. Stem cell therapies we are developing strategies to correct autologous DMD stem cells with a lentiviral vector. We will assess safety and efficacy using myogenic stem cells injected into a single human muscle. We will develop a safe, efficient method to transduce stem cells for systemic delivery.

6. Experimental medicine exercise physiology/therapy we will exploit the critical mass of expertise and new experimental exercise facilities we established with £2m host investment support across UCL and NCL. We will address key experimental questions in relation to molecular basis of exercise benefit and identify genetically stratified NMD groups for whom exercise is safe and effective.

7. Industry partnerships continuing strong industry partnerships will enable us to i) Develop and apply new experimental therapies e.g. antisense ii) Reprofile licensed drugs e.g. bezafibrate in mitochondrial disease and retigabine in channel disease iii) Develop MRI biomarkers iv) Use industry compound libraries to screen preclinical NMD models. Successful partnerships already exist with GSK; Prosensa; AVI, Shire, Senexis; Santhera, Trophos.

Funding requested from the MRC is £3.3 million. In addition, we have agreement for a further **£3.6 million of new host support** and agreed **£750k of new industry** support over the period 2013-2018.

2. Importance of UK Translational & Experimental Medicine Research in Neuromuscular Diseases

Unmet UK health burden of neuromuscular diseases

Neuromuscular diseases (NMD) are an important group of disabling conditions caused by impairment of peripheral nerve and/or skeletal muscle function causing premature death or major chronic disability, which may be compounded by cardio-respiratory involvement. Genetic examples include muscular dystrophy (~1 in 3500), Charcot Marie Tooth (CMT) neuropathy (~1 in 2500), channelopathies (~1 in 50-100,000) and mitochondrial diseases (~1 in 5000). Acquired examples include chronic inflammatory demyelinating neuropathy (CIDP) (~1 in 1500) and inclusion body myositis (IBM) (~1 in 10-50,000). NMD represent an important unmet health burden for the nation. This is despite the excellent clinical infrastructure provided by clinical centres and the nationally commissioned NHS funding for care and diagnosis of rarer NMD lead by MRC Centre PI's (e.g. mitochondrial disease, congenital muscular dystrophies & myasthenia and muscle channelopathies).

How the Centre fills a strategic need and how it has developed its approach to meeting the original mission. In 2006, we identified a lack of national strategic focus to enable translation of science into patient benefit. There had been significant progress in NMD discovery science, frequently led by internationally high profile UK clinicians and scientists, but translation into patient benefit had been disappointing. The UK risked falling behind other countries such as France, Germany and the USA who had established nationally funded systems to support the NMD translational pipeline. UK progress was also hindered by a notable absence of a NMD experimental medicine trials culture. This was in sharp contrast to standard cancer clinical practice in which patients were routinely offered entry into registries, cohorts and experimental trials. **This MRC Centre, which has encapsulated a highly successful London-Newcastle collaboration, has led the UK efforts to link discovery research to experimental medicine in the last four years** via the initiation of **six core activities** specifically designed to help overcome translation "gap-1" from discovery science into experimental medicine:

- **Core-1 Stratified cohorts for personalised medicine**: development of highly phenotyped genetically stratified patient cohorts as an essential prerequisite for personalised medicine.
- **Core-2 Experimental trials support**: a system of coordination and support to link discovery science to innovative experimental trials in the five neuromuscular disease themes.
- **Core-3 Neuromuscular human cell biobank**: a resource of human fibroblasts/muscle cells for preclinical testing and discovery science linked to routine NHS diagnostic biopsy procedures.
- **Core-4 MRI biomarker outcome measure development**: physics development and systematic application of quantitative MRI as a biomarker and NMD outcome measure.
- **Core-5 Capacity building for future NMD translational research**: education and capacity building PhD programmes in NMD translational medicine.
- **Core-6 Animal NMD models**: improved linkage and collaboration between expert NMD clinical and animal scientists to evaluate validity & translatability of findings in animal models to humans.

The core activities were critical in adding value to discovery science and enabling delivery of experimental medicine studies in each disease theme: i) Dystrophin restoration in DMD[1], ii) Mexiletine in

muscle channelopathies[3], iii) Vitamin C in Charcot Marie Tooth 1A[10], iv) Heat shock protein upregulation in inclusion body myositis[12], v) Resistance and aerobic exercise in mitochondrial disease[13], vi) Idebenone in mitochondrial disease[14]. The following case study provides one typical example of how the Centre added value and used the biobank facilities, stratified cohorts, MRI protocols, and experimental trials support to deliver a new DMD experimental study in London and Newcastle:

MRC Centre DMD exon skipping study: *The AVI-4658 study, co-funded by MRC & AVI BioPharma, recruited 19 boys with exon 51 eligible deletions. Its design was based on a previous proof of principle study which had utilised MRI as a measure of muscle damage. Recruitment occurred ahead of schedule and MRC Centre support enabled a) rapid access to large number of patients followed in London and Newcastle; b) effective links to UK Action DMD-TREAT-NMD registry; c) rapid interrogation of North Star UK DMD cohort, with longitudinal clinical functional data from >500 DMD boys. The MRC Centre infrastructure allowed us to contact and recruit boys who met strict entry criteria very rapidly. The MRC study coordinators were instrumental in setting up the study at London and Newcastle, coordinating rapid recruitment and maintaining the MRC cohorts. They also arranged research update newsletters for patients, transport and reimbursement. After consent, each patient underwent a skin biopsy to assess efficacy of antisense in inducing exon skipping in vitro. Fibroblasts were grown and stored in the MRC Biobank, where the research muscle samples were also stored after completion of the project. This proof of principle study confirmed dystrophin upregulation in vivo*[1]*. In renewal, all Centre core activities will enable delivery of new antisense chemistry with industry.*

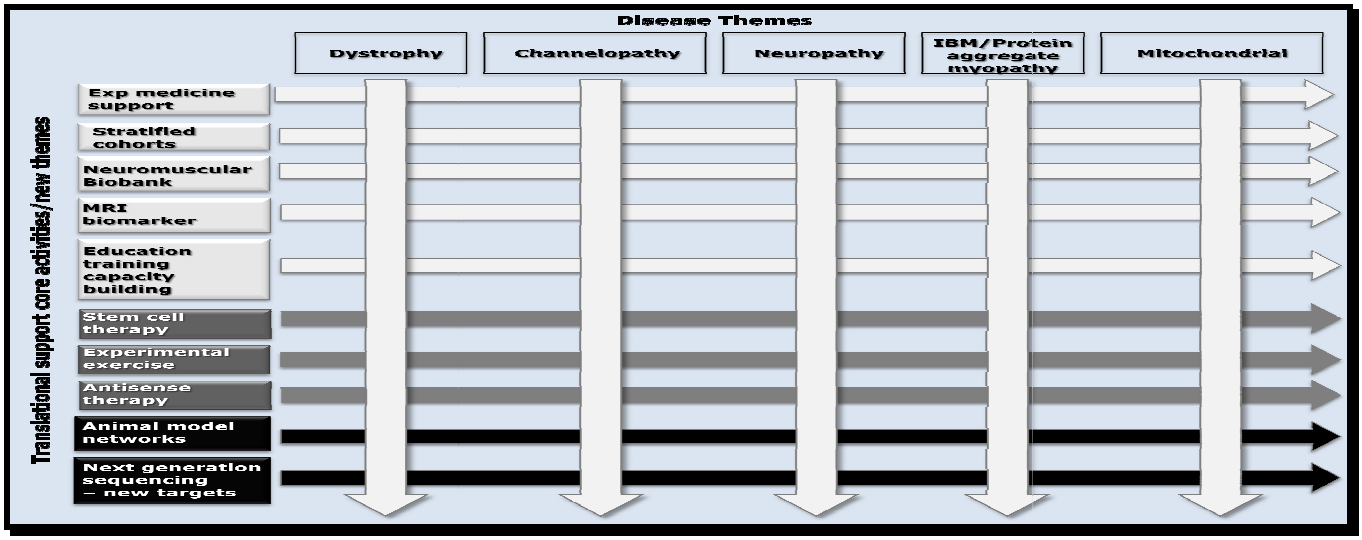
The primary focus of the MRC Centre in the next funding period is to embed and maximise the added-value of the Centre core activities to deliver more "pull through" of discovery science to new experimental treatments in each of five disease themes: muscular dystrophy, neuromuscular channelopathy, neuropathy, inclusion body myositis and mitochondrial disease. Core activities will enable us to maximise the potential of three important developments:

- Discovery science progress has improved pathophysiological understanding and identified more targets for pharmacological/genetic intervention. Progress will accelerate with next generation sequencing (NGS) DNA/RNA technology.
- Availability of animal models for preclinical optimisation of targets for "personalised" subsets of genetically stratified patients.
- Increased industry interest in rare disorders because of favourable regulations for orphan disease drug development and industry increasingly uses rare disorders as a route to market.

The **six MRC Centre core activities** will be developed and refined in the next funding period to maximise added value to separately funded (>£60m) programmes of discovery science lead by Centre PI's (examples of discovery science are in section 3 and in appendix II). ***Matching 1-1 support in each core activity demonstrates very major UCL & NCL host commitment to a renewed MRC Centre.***

Figure 1 illustrates the Centre's five vertical disease themes and the interdependence of disease themes and core support areas: each theme has critical mass of discovery science supported by major programmes of separate funding (>£60m) that underpin the Centre's translational activities. The ten left-hand column boxes represent cross cutting themes that are the key tools of the Centre designed to add value by aiding and informing translation of discovery science into experimental patient studies. The top five light grey boxes on the left indicate the ***core activities that are supported by the requested MRC funding (matched by host support)*** and that cut across the vertical disease themes overcoming obstacles to translation into man. Also shown in this diagram are the new Centre experimental therapy directions in which the Centre has developed critical mass (three light grey boxes represent new therapy themes: stem cell therapy, experimental exercise therapy and antisense therapy). These therapy themes are separately funded, but major added value is achieved by linking with the Centre core activities. Finally, there are major independently funded programmes of next generation sequencing (NGS) and animal model research (shown as left hand column black boxes). NGS in the Centre has already lead to the discovery of new genes[16] and new therapy targets by Centre PI's, and this will likely increase in renewal. *(Please note: full details of all core activities, including all related outputs for the past four years, and other additional useful reviewer information, such as previous SAB reports, is available at a* ***dedicated MRC Centre renewal reviewer web page at:*** **http://www.cnmd.ac.uk***)*

Figure 1.



MRC Centre core activities and importance for experimental medicine

The Centre will continue to drive UK NMD experimental medicine by evolving the six core activities:

1. Centre core activity 1 - MRC Centre stratified cohorts for personalised medicine: an essential activity of the MRC Centre in renewal is to support and maximise the potential of the UK **MRC Centre stratified patient cohorts**. The MRC Centre patient cohorts, whose core diagnostic data are being aligned to stored biomaterials and accurate contemporary natural history studies, are crucial to deliver translational medicine. We are at the forefront of such efforts internationally. Samples of patients studied with targeted genetic based therapies can provide valuable correlates with treatment response and ultimately biomarker validation. Patients with rare conditions, meeting stringent inclusion criteria, can be identified rapidly for recruitment into experimental studies. Such cohorts are the basis for proof of principle studies of potential therapies in the preclinical phase (e.g. recent/planned Centre studies of antisense in Duchenne); they are the source of patients for definition of appropriate outcome measures and ultimately for early and late phase studies. Over the previous funding period we placed a high priority on the consolidation of clinical datasets, the extension of these datasets and co-ordination of registry and natural history cohort activity, all in relation to the stored samples in the MRC biobank. This activity enabled and linked with other investments: the MRC funded Centre mitochondrial cohort programme, the EU FP6 funded Network of Excellence TREAT-NMD; the EU FP7 funded BIO-NMD initiative and UK patient organisation funding e.g. Muscular Dystrophy Campaign (MDC). Our cohort work is a major collaboration with patient organisations and represents a very strong area of MRC Centre public engagement. We have started to link clinical data, patient level functional/outcome data, genetic data, biopsy data and biobank data for individual cases. The aim in renewal is to achieve a full integrated database combining all above data streams at the single patient level. Such a fully developed, internationally unrivalled, MRC Centre integrated stratified patient cohort database will drive personalised experimental medicine and collaborations internationally and with industry. **The MRC Centre linked cohorts now contain over 2000 patients across the five disease themes**. Examples of the experimental medicine studies they will support are provided in section 3 below.

i). Muscular dystrophies (DMD, congenital muscular dystrophies, LGMD, FSHD, myotonic dystrophy): Centre investigators (FM, MH) lead on national natural history datasets (n>500) and on co-ordination of TREAT-NMD international patient registries (HL). (See http://www.cnmd.ac.uk).

ii). Neuromuscular channelopathies: Centre investigators (MH, HL, JP, DK) coordinate nationally commissioned channelopathy diagnostic services with >850 patients in the channelopathy cohort.

iii). Inherited neuropathy cohort: Centre investigator (MR) leads the MRC Centre cohort (n>1000) and co-leads the NIH inherited neuropathies consortium (http://rarediseasesnetwork.epi.usf.edu/INC/). Over 200 deeply phenotyped MRC Centre cases entered into the MRC-NIH natural history collaboration.

iv). Inclusion body myositis cohort: Centre investigators (MH, DT, JM, HL,) in collaboration with Oxford (DHJ) established a prospective cohort of IBM patients (n=164).

v). Mitochondrial disease cohort: the MRC Centre was crucial to a successful bid to the MRC Translational Medicine Board for funding the MRC Centre Mitochondrial Disease Patient Cohort (UK). This collaborative study (UCL, NCL & Oxford) includes >850 patients with a biochemical or genetic

diagnosis and detailed clinical assessment, and is the largest mitochondrial patient cohort world-wide generated through active collaboration and partnership across the MRC Centre.

2. Centre core activity 2 - MRC Centre experimental medicine support

We delivered a **ten-fold increase** in early phase experimental medicine clinical trials and natural history studies [2007 n=3; 2011 n=34] (see http://www.cnmd.ac.uk). There are > 200 NMD adults and children either currently involved in or who have completed MRC Centre experimental trials (including genetic therapy e.g. antisense[1], PTC 124, reprofiled drugs e.g. mexiletine[3] or new compounds e.g. arimoclomol/idebenone)[12,14] compared to <30 when the Centre commenced [2007 n=29; 2011 n=241]. There are now many additional funded studies at both early and advanced planning stages detailed in the scientific plan section 3 below, and also in the Centre Science programmes in appendix II. As detailed in the case study above, the MRC Centre translational support core activities remain an essential prerequisite for these studies at many levels, including trial co-ordination support and "know-how". There are three new therapy themes; i) **Antisense** (described in section 3 & in appendix II), ii) **Stem cell therapy** (described in section 3 and in appendix II) iii) **Experimental exercise therapy** (described in appendix II).

3. Centre core activity 3 - MRC Centre neuromuscular cell biobank

The MRC Centre biobank has exceeded all milestones and provided valuable human muscle cell cultures for discovery research and preclinical therapy testing. Any child or adult undergoing an NHS diagnostic biopsy in UCL or NCL can now consent to donate part of the diagnostic sample to the MRC biobank. Currently, the MRC biobank has built a collection of > 1870 anonymised samples linked to patient data using a biobank ID and secure database. These include mostly myoblasts and fibroblasts but also urine, whole blood, serum and plasma. Patient data are updated against genetic reports. All cultures are mycoplasma tested. All myoblasts are tested for myogenicty and differentiation to myotube by fusing and desmin staining. More than 1000 lines have been shared with nine UCL groups, five NCL groups, and more than 50 groups outside the MRC Centre, confirming the national and international science resource we have created. In addition, more than 1000 frozen muscle biopsies are stored with consent for research in the UCL and NCL diagnostic pathology labs. Samples are distributed under a strict governance framework, with an institutional MTA, and only after having verified the ethical approval of the recipient lab for the planned study. The MRC Centre biobank is fully included in international biobanking frameworks such as EuroBioBank and BBMRI. **We will build on the success of the biobank in renewal and will:** i) Increase sample size by continuing routine collection of myoblast and/or fibroblast cells from each patient undergoing diagnostics and extend sample collection to other UK centres, ii) Systematically collect biomaterials from each patient enrolled in clinical trials or natural history studies at the Centre or collaborating partners (e.g. exon skipping in DMD; Jain natural history for LGMD2B; FOR-DMD steroid trial), iii) Introduce immortalisation of targeted myoblast samples using recently developed method (retroviral transduction with both telomerase and cyclin-dependent kinase 4 expressing vectors) with French collaborators at the Institute of Myology, iv) Introduce immortalisation of targeted fibroblast samples using the method previously used by NCL PIs (retroviral transfer of papillomavirus E6E7 genes) v) Introduce MyoD-transfect human fibroblasts to generate myogenic cells for selected patients where muscle biopsies cannot be obtained, vi) Collect muscle derived stem cells vii) Introduce additional measures of quality control (ISO certification). viii) Increase collection of fibroblasts from neuropathy patients to be available for separate studies to generate iPS cells. Studies to be supported by the MRC biobank are described in the science section 3 below and in appendix II.

4. Centre core activity 4 - MRC Centre MRI biomarker platform for the quantification of nerve and muscle pathology: the central aim of the **MRI platform** activity is, through methodological advances and systematic patient studies, to develop MRI measures of NMD activity and progression, applicable as timely, practical outcome measures in both single and multi-centre clinical trials. MRI will provide key non-invasive readouts underpinning new clinical studies within the five disease theme programmes of the Centre. We have developed MRI methods to reliably quantify muscle pathology as potential outcome measures in both primary myopathies and peripheral neuropathies in adult and paediatric patients. The use of 'Dixon' fat-water MRI to quantify muscle fat infiltration, a common pathological manifestation across the NMD, has been a unifying theme. We established a **platform infrastructure** across the Centre to enable this research. In London, through the MRC Centre, we established the UCL Neuromuscular MRI Research Group (www.ucl.ac.uk/neuromuscular-mri). In NCL, the existing experience of the MRI Centre (www.ncl.ac.uk/magres/) in assessing muscle pathology by MRI and spectroscopy was, through the support of the MRC Centre, extended to muscular dystrophies and cardiomyopathies. Cross-centre collaborations established the following **standardised methods and protocols** that can now be used as outcome measures in clinical trials: i) Quantitative Dixon lower-limb

fat-water methods optimised in Newcastle (Philips) and London (Siemens)[17.18], ii) Magnetization transfer (MT) MRI correlated with muscle strength in CMT1A supporting MT-MRI as an outcome measure[19-21], iii) innovations to improve MR sensitivity[19-21] iv) IDEAL-CPMG, which provides in a single scan muscle water and fat content and respective T_2 relaxation times[22], v) validated protocols for multi-parameter quantitative lower-limb muscle MRI with test-retest and normal age-dependence data, vi) Quantification of sciatic nerve hypertrophy, a potential disease marker in CMT1A[23], vii) quantification of early changes in cardiac structure, wall motion and energetics through the development of cardiac wall tagging and cardiac phosphorus spectroscopy, viii) the use of phosphorus magnetic resonance spectroscopy to detect metabolic abnormalities at rest and under exercise in skeletal muscle. The methods and protocols were applied in various **Patient Studies:** i) Multi-centre study involving Newcastle and London assessing quantitative MRI in Limb Girdle Muscular Dystrophy 2I (LGDM2I)[17,18]; ii) Cross-sectional assessment of the localised mechanism of cardiac involvement in LGMD2I; iii) the identification of non-invasive metabolic biomarkers in skeletal muscle at rest and under exercise in LGMD2I; iv) a cross-sectional and longitudinal MRI investigation in inclusion body myositis (IBM) and Charcot-Marie-Tooth disease (CMT1A); v) Muscle histology vs. MRI in Duchenne muscular dystrophy[24]. Recently initiated/planned funded studies include: i) MRI in progressive external ophthalmoplegia; ii) cross-sectional evaluation of MRI in non-dystrophic myotonias, iii) MRI in inflammatory neuropathies iv) MRI to assess progression and therapy in periodic paralysis. The significant imaging and spectroscopy expertise in NMD across the Centre MRI groups has resulted in close **Industrial Links** with GlaxoSmithKline, Prosensa, AVI and Genzyme. Collaboration agreements including NMD MRI development are in place with Siemens and Philips. **Over the next five years** we will deploy MRC Centre-established MRI protocols in patient experimental medicine studies in the five disease themes and continue technical development to fully exploit MRI to provide non-invasive, objective readouts. We will extend anatomical coverage (upper limbs; diaphragm; 'whole-body' coverage), develop improved image segmentation and data analysis methods. We will investigate the potential of muscle diffusion-tensor imaging and apply multi-parametric methods to probe intra- and extramyocellular water and lipid distributions. A priority will be to establish MRI measures in pre-fatty infiltrated muscle where therapies may be most effective. We will develop high resolution 'whole-nerve' imaging and segmentation to quantify gross morphological changes, and through quantitative MRI assess the potential of "virtual nerve biopsy"; determining measures that relate to axonal loss, demyelination, inflammation, and conduction block. We will continue essential work to develop standardisation and validation of imaging methods and protocols across both MRC Centre sites and link with NMD centres across Europe & USA.

5. Centre core activity 5 - MRC Centre capacity building and training (see section 4 below).

6. Centre core activity 6 - MRC Centre NMD animal model translation strategy: many MRC Centre PI's have significant programmes of NMD animal model research (see other support). There are strong links with MRC Harwell (EF), and membership of the recent successful MRC mouse network bid (DT, FM, EF, LG, MR, MK, MH). Centre PI's agreed it was not realistic for the renewal to resource major animal research programmes. However, there are very important opportunities for us to add value to existing MRC investments by linking human clinical scientists and human cohorts with animal scientists and animal models to drive translation through, for example, informing animal model preclinical therapy assessment (also strongly highlighted by our SAB 2009 and 2011 who said *"the Centre is virtually uniquely placed to generate effective "cross talk" between NMD animal experts and clinical scientists"* - see SAB reports appendix III). The MRC Centre has a four-fold strategy to add value, support and inform existing MRC investments in NMD animal model research: i). A series of translational PhD projects in the Centre which utilise preclinical animal models in each of the major disease themes, ii). MRC Centre PI's will play an active role in optimising the success of the recently awarded MRC Neuromouse Consortium and build further links with MRC Harwell, iii). Centre PI's and PhD students will continue to utilise the successful MRC Centre NMD animal phenotyping laboratory established using the original Centre award allocation (50k). iv). We will establish a series of innovative national MRC Centre NMD animal-clinician scientist workshops with MRC Harwell to maximise "cross talk" and joint new projects between human and animal scientists. *The overarching aim of our animal model strategy, linked to other supported programmes, is to understand how reversible functional impairment turns into irreversible structural deficits and how experimental therapy can prevent, delay or compensate this*. This will be studied in three areas, namely: *(i) which factors control axonal transport and Schwann cell phenotype; (ii) which mechanisms dominate transition from ion channel abnormalities to structural change; (iii) how can better understanding of experimental therapy be harnessed for human trials,* (more details of the three areas provided in the animal model science theme appendix II).

3. Scientific plan

The scientific programmes that underpin and gain value from the Centre's core translational activities attract >£60m of separate grant income (see other support). Here we provide examples from four of the disease themes, illustrating how the Centre's core areas have added value to discovery science programmes, and we outline experimental medicine plans (details of the science in the fifth disease theme, IBM, are included in appendix II). *Each scientific programme will offer range of PhD projects.*

A. Muscular Dystrophy-discovery science and experimental medicine:

UCL lead: Francesco Muntoni (FM) NCL lead: Katie Bushby (KB),

Background/importance: muscular dystrophies cause progressive weakness and disability often with cardiorespiratory complications. Duchenne muscular dystrophy (DMD) is the most frequent childhood form, causing death in teens or early adult life. A high de-novo mutation rate means genetic counselling alone will not reduce incidence. Centre PIs also have strong expertise in other areas of dystrophy research. These include dystroglycanopathies, with an active programme of gene discovery (recent discovery of three novel genes), mouse model phenotyping and translational research using various genetic therapy and small molecule approaches, discussed in appendix II[25]. Another area of strength includes the investigation of the therapeutic potential of stem cells in dystrophies (below and in appdx II).

Discovery science and experimental medicine we delivered

Antisense oligonucleotides (AOs) in DMD: this is an area of strong and effective multidisciplinary collaboration across the MRC Centre with multiple research projects involving clinician scientists and researchers. Centre investigators (FM, JM, KB, VS) have successfully completed a recent MRC funded phase IIa study using a morpholino (PMO) AO developed by the UK MDEX consortium to skip exon 51 (http://www.mdex.org.uk/ PI-FM) in collaboration with an industrial partner AVI BioPharma[1]. This experimental study followed the preclinical optimisation and identification of the lead AO compound for skipping exon 51 using muscle cells from the MRC Centre biobank[26,27].

New dystrophy discovery science and experimental medicine studies

There are major opportunities to take dystrophy discovery science into man over the next 1-5 years. These opportunities will be accelerated by the substantial value the MRC Centre can add in the areas of stratified cohorts, neuromuscular biobank & MRI biomarker outcome development. There are unrivalled translational research training opportunities. Several active projects are already at the in-man stage; others are currently being pursued at a preclinical level in cellular or animal models and will benefit from the carefully phenotyped and genotyped Centre cohorts.

Antisense oligonucleotides (AOs): MRC Centre investigators (FM, KB, VS) have obtained funding for collaborative industry sponsored studies with GSK/ Prosensa including a phase IIb study using a 2'OME backbone AO designed to skip exon 51. A further study on non-ambulant older DMD patients who could benefit from exon 51 skipping is at the planning stage using 2'OMe AO. In collaboration with Prosensa we are also planning a phase I study of two novel 2'OMe AOs that target exons 45 & 53. MRC Centre investigators in collaboration with the MDEX consortium have identified a lead morpholino AO sequence to skip exon 53[28], which has been validated using patients' cells stored in the MRC biobank, and has undergone patent protection (a grant for an investigator-led phase I/IIa study being submitted). This will be a randomised placebo controlled dose escalation study and will also investigate the role of non invasive biomarkers (muscle MRI and serum miRNA) in monitoring disease progression and therapy response. We have just secured an Association Francaise Myopathies (AFM) consortium grant to develop outcome measures for non-ambulant DMD. This AFM grant will support an investigator-led collaborative clinical trial (in UCL, NCL and Paris) to assess safety of PMOs in non-ambulant DMD and to assess the value of the exploratory outcome measures being developed. In addition, this grant is focused on identifying safety and efficacy biomarkers to evaluate the effect of chronic PMO administration and determining optimal protein and RNA techniques to quantitatively assess efficacy of this genetic intervention. MRC centre PIs and the MDEX consortium also obtained a Wellcome Trust Health Innovation Challenge Fund grant, in which a new generation AO (a PMO linked to a peptide for improved skeletal and cardiac muscle targeting) is currently undergoing efficacy and safety testing in the mdx mouse model. The expectation is that a safe compound that is more efficient than current 2'OMe and PMO AOs will be found and we will proceed to a phase I trial in DMD boys eligible for exon 53 skipping using a peptide modified PMO. Currently, nine classes of peptide conjugations to the PMOs are being investigated by the Oxford and Cambridge members of the MDEX consortium and their efficacy is tested in vivo in the mdx mice in the MRC Centre. Significant recent consortium progress has identified peptides that improve skeletal and cardiac muscle PMO targeting *by more than ten-fold*. Preclinical studies will investigate route and dose optimisation including repeat low doses and assess biodistribution relevant for skeletal and heart muscle. Correction of heart dystrophin expression and function in mdx

mice will be assessed using cardiac conductance catheter techniques which are available in the Centre in NCL (VS). Some of these peptide modified PMOs also appear to be able to cross the blood brain barrier. Preclinical toxicology of the lead compound is expected in 2013. In other preclinical developments, collaborative work between JM FM and SH is also assessing the role of nanoparticles to improve AO uptake in tissues which are not targeted by the current AO chemistries such as cardiac muscle and brain. Both this and the peptide modified PMOs might have important implications for other conditions, such as spinal muscular atrophy, in which clinical applications of AOs are being planned in USA (ISIS) using a MOE backbone AO. Investigators in the MRC Centre also have expertise in this condition both from the clinical perspective and the modification of splicing to retain SMN2 exon 7[29]. As discussed in appendix II, MRC Centre investigators recently identified a novel AO sequence which is very effective in improving life expectancy in a transgenic animal model of severe SMA.

Other therapeutic approaches: **stem cells**: PIs of the Centre (UCL:FM, JM, AT,OD, NCL: HL) are undertaking an MRC funded translational research project to identify an optimal stem cell[30]; an efficient and safe lentiviral vector[31]; and an "opti-dystrophin" construct containing all the necessary regulator elements, including the nNOS binding site. We aim to perform a proof of principle study comparing a local injection of lentivirally-induced opti-dystrophin expressing autologous stem cells into a single human muscle. These stem cell projects will link to the expertise of Giulio Cossu who, thanks to UCL host support, joins the Centre in 2012. Cossu's group will also pursue their pioneering work on the use of human artificial chromosomes (HACs) containing the whole dystrophin locus as a potential DMD therapy. Their recent work has shown that the dystrophin HAC (DYS-HAC) has efficacy in a dystrophic mouse cell model[32]. In renewal, efforts will focus on generating HACs which will be transferred into human dystrophic mesoangioblasts which will be challenged for their ability to repair dystrophic muscle and ameliorate the disease. The translation of this alternative strategy to trials will be greatly supported (value added) by the Centre experimental trials/cohorts core support (see appendix II). **Regarding gene therapy**; Centre PIs have recently initiated a collaboration with Genethon and Paris (Thomas Voit, Institute of Myologie) to recruit DMD boys with deletions eligible for exon 53 skipping in order to undertake a detailed function and MRI assessment of upper limb weakness progression. This is in preparation for a phase I study of an AAV vector expressing antisense sequences linked to a modified U7 small nuclear RNA. The vector will be delivered by perfusing a single upper limb in the first instance.

How the MRC Centre will add value to future studies: the Centre has been instrumental in delivering completed studies and will underpin planned experimental trials. The main added value relates to biobank, muscle imaging, experimental medicine support and stratified cohorts. **MRC Centre Biobank**: the biobank will be instrumental for continuing to optimise new target sequences for skipping other exons (or including exons in SMA). The biobank will also support studies on DMD stem cells; the dystroglycanopathy studies; and high throughput next generation sequencing studies on muscular dystrophies. **MRC Centre MRI biomarker development**: we will investigate the role of muscle imaging and spectroscopy using MRI in detecting changes related to the administration of AOs in mdx mice. This study will assess both short term effects of AO in reducing muscle inflammation and longer term effects on muscle anatomy. A dose response on MRI with different AO regimens will be assessed and correlated with the level of dystrophin in mdx muscle, and with serum biomarkers. Studies performed in NCL by VS will evaluate the effects of AO on cardiac function in mdx mouse models of DMD. These mdx mouse MRI studies will be paralleled by natural history studies of muscle MRI changes in DMD boys at different level of functional abilities, performed both in collaboration with industrial partners (Prosensa; GSK) and academic sponsors (MRC: Genethon). These studies will inform planned intervention studies using AOs to skip various exons in different DMD cohorts. **MRC Centre stratified cohorts**: all the studies above on exon skipping in DMD will benefit from the stratified MRC Centre supported North Star cohorts both for recruiting patients with eligible deletions and assessing inclusion criteria, and to assess and validate novel outcome measures relevant for non-ambulant DMD individuals. Indeed, these novel AO therapies are **highly personalised genetic therapies** and only a subgroup of DMD boys with eligible deletions can be recruited into such specific studies. **MRC Centre experimental medicine support:** the MRC trials coordinator will continue to be central to maintaining the stratified cohorts for personalised medicine and streamlining regulatory support and trial monitoring and delivery. These cohorts could also be instrumental for future post-marketing surveillance studies. In addition, maintaining accurate information on patients followed at the Centre will be essential for the efforts of various PIs in identifying novel genes. In this respect, a recent collaboration between FM and the Sanger Centre in Cambridge has led in the last few months to the identification of four novel genes responsible for a dystroglycanopathy; two novel congenital myopathy genes and one gene for a distal motor neuropathy. The recent identification, as part of a separate collaborative study, of a gene responsible for a congenital

myopathy, the function of which appears to be that of regulating satellite cells myogenesis, highlights the cross links between different aspects of this research program[16,33]. These gene discoveries have revealed new therapeutic targets.

B. Inherited Peripheral Nerve Disorders: discovery science and experimental medicine

UCL lead: Mary Reilly (MR), NCL lead: Rita Horvath (RH).

Background/importance: The main inherited peripheral neuropathies are Charcot Marie Tooth disease (CMT), the distal hereditary motor neuropathies (HMN) and the hereditary sensory and autonomic neuropathies (HSAN). Severity varies, but patients most commonly have significant life-long disability.

What we have delivered and how the Centre will translate new discovery science into experimental medicine studies

Genetics: Using MRC Centre stratified cohorts, we identified many new causative mutations in a wide range of genes (e.g. Frabin, SH3TC2, TRPV4, Fam134B, PMP22, MPZ, MFN2). Based on MRC Centre cohort studies assessing mutation frequency in a UK population, we developed and implemented new genetic diagnostic algorithms for clinical practice.[34] Linked to the MRC cohorts we (MR,MH,JH,HH) secured new MRC and Wellcome funding (>£1m) for an Illumina platform next generation sequencer (HiSeq2000) to employ targeted & whole exome sequencing. We already identified a new gene causing the neuropathy Brown Vialetto von Laere syndrome (submitted), two new genes causing axonal CMT2 and also the FBXO38 gene in dominant HMN (submitted and see appendix II). In our MRC neuropathy cohorts, 45% of CMT2 cases and 80% of HMN and HSAN cases remain genetically undefined. ***In renewal***, new gene discovery using NGS will be an important activity. We could not do this research without maintaining/updating the MRC neuropathy cohorts (now >1000 patients). Developing links to patient biobanked fibroblasts (& generation of iPS cells) will be critical to enable functional studies of new genes. New gene discovery will, enable further genetic stratification, and reveal new therapy targets.

MRC Neuropathy Stratified Cohorts: the MRC neuropathy cohort enabled Centre investigators (MR, FM) to become leading partners in the international NIH (USA) funded inherited neuropathy rare disease consortium (RDCRC, MR is Co-Director). With new Centre investigator RH in NCL, we added 200 new NCL patients, further enhancing this national cohort for genetics, natural history studies and planned experimental trials. ***In renewal,*** MR, RH and JS will continue to deeply phenotype and stratify the cohort employing clinical, neurophysiological, genetic, MRI and functional techniques. We will take advantage of recent advances in techniques to phenotype sensory deficits (MK) and continue to develop MRI Biomarkers utilising the cohort.

Development of Outcome Measures: developing validated, sensitive and responsive outcome measures in neuropathies is crucial for powered trials. We (MR) published the first validated composite scale (CMTNS) used as an outcome measure in CMT trials. Based on outcome data analysis from the MRC Centre experimental trial of ascorbic acid in CMT1A we refined the sensitivity and reported CMTNS2[35]. The MRC Centre has enabled a major new direction for neuropathy outcomes research with development of quantitative muscle MRI as a biomarker of denervation. We developed a lower limb muscle MRI protocol in CMT patients (see MRI core activity earlier). Initial pilot cross sectional data indicates this MRI method is a more sensitive marker of early muscle denervation than current clinical or neurophysiological methods[19]. There is clear potential for MRC Centre MRI protocols to be biomarker outcomes in future neuropathy trials. ***In renewal*** we will i) study responsiveness of the new CMT scale (CMTNS2), ii) study the sensitivity/responsiveness of our new muscle MRI protocol in stratified cohorts of CMT, HMN & HSAN patients, iii) continue to identify more sensitive methods of measuring muscle power using isokinetic myometers (HUMAC) and compare with conventional MRC score methods.

Understanding Molecular Pathophysiology: better understanding of fundamental pathophysiology of neuropathy will help identify new treatment targets. Our discovery programme has produced new insights into disease pathogenesis. For example, we recently identified the first human null peripheral myelin protein (PMP22) patient and correlated human findings with the PMP22 null mouse. These data suggested PMP22 is especially important for cranial motor neurons and spinal sensory neurons in early development and differentially affects myelination between motor and sensory nerves[36]. We also defined the function of the protein SH3TC2 and showed how mutations result in mis-targeting of SH3TC2 away from the recycling endosome as the fundamental molecular defect leading to CMTC4C demyelinating neuropathy[37]. ***In renewal*** we will focus on four areas; i) inherited protein folding disorders of the PNS, ii) mitochondrial function in inherited neuropathies, iii) pathophysiology of hereditary sensory and autonomic neuropathy type 1 (HSAN1) and iv) animal models and axonal transport (see appendix II).

1) Inherited Protein Folding Disorders of the PNS From the MRC neuropathy cohort we recently identified mutations in the small heat shock protein genes HSPB1 & HSPB8 in a large series of dHMN

and CMT2 cases. To understand the pathogenesis of this important group of motor nerve disorders we linked with expert groups across the MRC Centre encompassing clinical inherited neuropathy (MR), molecular genetics (HH, MR) and animal and *in vitro* functional modelling (LG,EF). The functional group (LG,EF) are studying the pathogenesis of peripheral motor disorders using different models including: i) transgenic mice, ii) mice with endogenous mutations in genes associated with motor nerve degeneration, and iii) *in vitro* models of primary neurons virally transfected with relevant mutant genes. In order to examine sensory and motor systems in neuropathy mouse models, we will use the MRC Centre neuromuscular mouse phenotyping facility at UCL (LG, EF, MK). This facility has been used by MRC PhD students and investigators to analyse the phenotype of a number of mouse models of motor nerve disorders. We have also established confocal systems to study mitochondrial function and axonal transport. ***In renewal*** we will investigate how protein misfolding disorders cause motor nerve degeneration. We will initially focus on modelling our HSPB1 mutations, and in parallel we will study a recently generated knockout mouse (HSJ1) that develops a severe motor neuropathy. We will study axonal transport in axons of both primary motor neurons virally transfected with HSPB1 mutations and neurons from the HSJ1 knock out. We will also study axonal transport in motor neurons from a newly developed HSPB1 transgenic mouse in *(collaboration: L. Van den Bosch)*. We will evaluate experimental compounds including, arimoclomol, which augments the heat shock response (we have already obtained MHRA approval for arimoclomol and have undertaken human experimental trials in the Centre in IBM) and has potential to ameliorate the effect of HSPB1 mutations. We will assess the effects of arimoclomol in HSPB1 mutant motor neurone cultures and transfected cellular models. We will use patient derived fibroblasts (biobank) to generate iPS cell derived motor neurons and study axonal transport.

2) Mitochondrial function in Inherited Neuropathies: we recently identified mutations in the mitochondrial gene (ATPase 6) in CMT2 (~2% of cases) with a predominantly motor phenotype. Functional data confirmed pathogenicity and phenotypic severity correlated with blood mutant load (submitted). These data indicate ATPase 6 mutations are the second commonest cause of CMT2 after the nuclear mitochondrial gene MFN2 mutations which has important genetic diagnostic implications. By whole exome analysis we recently identified a new nuclear mitochondrial biogenesis gene in CMT2. In one family we observed that the co-inheritance of a known pathogenic mutation in the variable loop of a mitochondrial tRNA gene for serine markedly worsened phenotypic severity, suggesting an important nuclear-mitochondrial interaction. ***In renewal*** we will use mitochondrial expertise across the MRC Centre to study both mtDNA and autosomal mitochondrial genes in inherited axonal neuropathies. Our plans include: i) studying the effect of recently identified mtDNA and nuclear gene mutations on axonal transport using iPS cell models ii) pursuing our recent discovery by investigating nuclear-mitochondrial interactions using cybrid systems and by evaluating mitochondrial gene expression patterns (with DT) iii) use the MRC Centre mitochondrial cohort to assess neuropathy involvement in detail.

3) Hereditary Sensory and Autonomic Neuropathy type 1 (HSAN1): from MRC Cohorts we published the largest series of HSAN1 patients with *SPTCL*1 (subunit 1 of SPT enzyme) gene mutations. Recently, we discovered a new disease mechanism in which the SPTLC1 mutation leads to a change in substrate specificity of the SPT enzyme resulting in generation of neurotoxic deoxysphingolipids (DSBs)[11]. We recently discovered two novel SPTCL2 (subunit 2 of SPT) mutations causing HSAN1. A pilot study in our SPTLC1/2 cohorts shows DSB levels correlate with disease severity (unpublished). ***In renewal*** we will longitudinally assess whether DSB levels correlate with disease progression, and determine the best outcome measures for a planned trial of serine therapy (see below).

Clinical Trials: we recently completed a significant international experimental trial of high dose ascorbic acid (AA) in CMT1A[10]. MRC Centre cohorts allowed us to achieve full recruitment rapidly. Importantly, our study indicated that the AA efficacy reported in the CMT1A animal model was not reproduced in the human disease. The study provided important insights into outcome measure responsiveness. As part of the Centre exercise therapy theme we have started to investigate both resistance and aerobic exercise in inherited neuropathies. We showed that hip flexor fatigue limits walking in CMT[38] and have just completed a trial of the effect of increasing hip flexor strength on waking ability in CMT (data being analysed). ***In renewal*** we will undertake two initial experimental trials. First, a CMT aerobic exercise trial which we have designed in collaboration with NCL. Second, an experimental trial of serine therapy in patients with HSAN1 caused by *SPTLC1* or *SPTCL2* mutations.

How the MRC Centre core activities added value to future studies: all above studies have gained significant value from MRC Centre specialised experimental trials support, biobank access, stratified cohort development, MRI biomarker development and use of MRC centre mouse phenotyping facility. MRC Centre PhD students have undertaken neuropathy research. All planned studies will be catalysed by the MRC core activities.

C. Neuromuscular Channelopathies: discovery science and experimental medicine

UCL lead: Michael Hanna (MH), NCL lead: Hanns Lochmüller (HL).

Background/ importance: Genetic dysfunction of ion channels causes disorders with altered nerve and muscle excitability, impaired neuromuscular junction transmission or altered excitation contraction coupling. Clinical manifestations include neonatal myotonia and/or weakness that may be fatal, episodic and progressive muscle weakness, craniofacial and limb deformities, and cardiac arrhythmias[39].

Discovery science and experimental medicine we delivered

The MRC Centre channel group have taken advantage of Centre core activities to make discoveries:

Muscle channelopathy genetics: we comprehensively defined the genetic architecture of muscle channelopathies discovering large numbers of new pathogenic misense, nonsense and frameshift mutations in muscle voltage-gated ion channels (SCN4A, CACNA1S, KCNJ2, CLCN1)[5,40].We showed that large scale gene rearrangements and copy number variation in CLCN1 can cause severe drug resistant myotonia[41]. The Centre developed the world's largest genetically stratified muscle channelopathy cohort and linked it to our nationally commissioned diagnostic service as an invaluable platform enabling the MRC natural history and experimental medicine studies we delivered (MH)[3,42,43]. New genotype-phenotype relationships discovered include first descriptions of neonatal hypotonia and stridor with genetic sodium channel fast inactivation defects, guiding changes in clinical practice[44,45]

Muscle channelopathy pathophysiological mechanisms: we tested the possibility that the commonest muscle channelopathy, hypokalaemic periodic paralysis (HypoPP), is caused by loss of voltage sensor positive charge in either the sodium (Nav1.4) or Calcium (Cav1.1) channel. In our cohort of >80 cases we found almost all harboured mutations of arginine residues that predict a gating pore current, identifying this as a common disease mechanism[5,7]. We undertook a pharmocogenetic correlation study and showed that SCN4A or CACNA1S mutations that predict a proton selective gating pore current consistently respond to carbonic anhydrase inhibitors. In contrast, radical amino acid substitutions predicting a non-selective gating pore currents do not. This allows stratification and prediction of treatment response[6].

Muscle excitation-contraction coupling disorders: we (FM MD) delineated the role of dysfunction of the sarcoplasmic calcium release channel RYR1 in human disease, used cohorts to define the phenotypic range of mutations, and implicated mitochondrial dysfunction in the pathophysiology[46].

Congenital myasthenic channelopathies: we discovered a gene associated with congenital myasthenic syndromes (CMS) (HL). Most known CMS genes encode structural components of the neuromuscular junction (NMJ) but we discovered mutations of the *GFPT1* gene (encoding an amino sugar synthesising enzyme) caused CMS with tubular aggregates. The exact NMJ function of GFPT1 is unclear, but has important implications for understanding synaptic physiology[8]. We characterised key clinical features in a large cohort of GFPT1 patients in the Centre and with Oxford (DB, JP)[9].

Translational activities supported by the MRC Centre

Completed large multicentre natural history studies (NIH funded) in genetically stratified muscle channelopathies; in particular periodic paralysis and non-dystrophic myotonias. We defined precise natural history and outcome measures for experimental medicine studies[3,42,43].

Completed the first multicentre international randomised controlled experimental trial (NIH funded) in a muscle channelopathy: myotonia congenita[3]. We showed that reprofiling of the use-dependent sodium channel blocker mexiletine has a highly significant benefit ($p<.0001$) compared to placebo when assessed by validated patient reported outcome measure. This work has resulted in an orphan drug status application to the European Medicines Agency (EMA)[3].

Developed new in vivo diagnostic electrophysiological protocols **and techniques:** (including first-in-man muscle sarcolemmal velocity recovery cycle measurements)[47] to diagnose, direct DNA testing and stratify muscle channelopathy patients[4,47] .

Added value from the MRC Centre: All the above discovery and translational studies have gained significant added value from the MRC Centre through specialised experimental trials support, biobank access, stratified cohort development, MRI biomarker development, and training of the MRC Centre PhD students. All planned studies will be catalysed by the key core areas provided by the Centre.

New channelopathy discovery science and experimental medicine studies

Channel research benefits greatly from Centre core activities. Each project is associated with separate funding and multiple collaborations often bringing investigators outside the Centre into NMD research.

Discover new genes (MH, HH): 20% of patients with muscle channelopathies do not have mutations in known genes and undiscovered genes likely exist. MH ,MR, HH have MDC/MRC/Wellcome funding (>£1m) for next generation sequencing to perform whole exome analysis, and we have identified 64 genetically undefined families from our stratified cohorts for this purpose.

Investigate relationship between genotype and phenotype (MH HH): we will ask whether gene modifiers, differential allelic expression and other epigenetic mechanisms can explain the poorly understood relationship between genotype and phenotype (funded by the MRC and MDC). These observations will extend to asking how genotype predicts drug response (supported by MRC and NIH). For example, the optimal treatment of HypoPP is not known. Although acetazolamide is sometimes effective in reducing attack severity, analysis of the MRC channel cohort showed ~50% of genetically proven HypoPP cases do not respond[6]. We found that patients with arginine to histidine substitutions in the voltage sensor region are more responsive to acetazolamide therapy than other mutations[6]. Indeed, we never observed a beneficial response with the R528G or R1239G substitutions in CACNA1A or with R672G in SCN4A. Thus our genetically stratified database has provided insight into the mechanism of this important drug. Further work will ask how variation in genes can impact drug response.

Understand the molecular pathophysiology of diseases caused by gating pore currents (MH, SS, RM, DK)**.** An aberrant gating-pore leak introduced by CACNA1S or SCN4A mutations has been proposed to be critical to the pathophysiology of HypoPP[48,49]. MRC Cohort analysis enabled us to provide compelling genetic evidence supporting this hypothesis[5]. However, it is unknown how this leak explains the tendency for hypokalaemia to trigger paralysis or how it relates to progressive muscle degeneration. Our genetic study (see above) showed that the response to acetazolamide correlates with the cation selectivity of the predicted gating pore current: when the S4 arginines are substituted by histidines, the gating-pore current is highly proton selective and the disease responds to treatment, whereas other substitutions that lead to a non-selective gating-pore current are treatment unresponsive. Thus our genetic findings strongly implicate the trans-membrane proton gradient as central to the acetazolamide response. With this insight we will use carbonic anhydrase inhibition as a tool to understand how the cation shunt results in paradoxical depolarisation of the muscle fibre. This will also be informed by our recent genetic discovery in the MRC cohort that a new mutation affecting a negatively charged residue in S2 of NaV1.4 causes HypoPP. A potential unifying explanation is that this residue also lines the gating pore and possibly interacts with S4, and that its neutralisation allows a leak current through the pore.[50] This idea is supported by very recent NaV1.4 x-ray crystallography data[51]. We will test this hypothesis directly by measuring the gating pore current in vitro and relate the findings to other gating pore mutations in SCN4A and CACNA1S. These studies will be done in xenopus oocytes and also in mammalian cell lines, allowing channel function assessment at physiological temperatures. Human myoblasts from the MRC biobank are also being explored for functional characterisation, and in the case of CACNA1S some mutations are available in mouse strains (see studies of muscle degeneration below), and we are already refining patch clamp recording methods from acutely dissociated mouse muscle fibres. These recordings will allow assessment of the channel function within the native context of functional muscle fibres. We will also examine the contribution of ATP-gated K^+ (K_{ATP}) channels to the effect of lowering the extracellular potassium concentration on membrane potentials. This channel is highly expressed in muscle fibres and its conductance exhibits an anomalous dependence on extracellular K+ concentration. Reduced K_{ATP} channel expression has already been shown in some patients with HypoPP[52]. We will ask if this is a general phenomenon and whether it depends on the nature of the gating-pore or other mutations. This work will improve pathophysiological understanding and may create druggable opportunities to test in genotyped patients.

Mechanisms of muscle degeneration in channelopathies (MH, MD): most patients with muscle channelopathies develop a severe myopathy but the mechanism is unknown and there is no treatment. We suspect calcium physiology is detrimentally altered by aberrant membrane excitability and there may be druggable opportunities. By extensively characterising the calcium physiology of cultured myoblasts from healthy human controls and biobank myoblasts from genotyped individuals with muscle channelopathies, and by evaluating calcium physiology in mouse models, we aim to identify new pathways implicated in channel myopathy. We have access to a knock-in mouse model of hyperkalemic periodic paralysis (Scn4a^{1592}) which has already shown a beneficial role of increased extracellular calcium and detrimental role of impairment of the sodium/potassium pump in myopathy development[53]. We will also study mouse models of HypoPP caused by mutations of CACNA1S (Cacna1s^{1239}, Cacna1s^{528}). We will compare the calcium handling of cultured myoblasts from this mouse with those of genotyped individuals with muscle channelopathies (MRC biobank) and healthy human controls. We also developed a method of studying calcium physiology in single muscle fibres, and will measure the resting [Ca^{2+}], characteristics of any spontaneous [Ca^{2+}] signals, and SR calcium release, which will be evoked by caffeine, by membrane depolarisation and by stimulation with acetylcholine.

Muscle channelopathies and new experimental medicine studies (MH, MK, DK)**:** the Centre has enabled us to build a genetically stratified cohort of channelopathy patients for natural history

experimental medicine studies already delivered[3,42,43]. Our recently completed experimental RC trial of reprofiled mexiletine established it is effective for many patients with myotonia congenita but ~30% remain drug resistant or tolerate mexiletine poorly[3]. Our cohort analysis has established that 50% of patients with HypoPP do not respond to carbonic anhydrase inhibitor therapy and this relates to genotype[6]. We will reprofile lacosamide, a novel anti-epileptic drug, that acts on sodium channels, and retigabine (which opens potassium channels) in genetically stratified mexiletine-resistant myotonia congenita patients. We will also test a novel KATP channel opening agent in the subgroup of acetazolamide-resistant patients with hypokalaemic periodic paralysis who have non-selective gating pore current S4 mutations. We will conduct these studies by combining our existing patient reported outcome measures (developed in the mexiletine trial[42,43]) together with new muscle MRI biomarker secondary endpoints. Recent pilot work indicates MRI detectable reversible muscle water accumulation correlates with weakness in HypoPP patients. We will ask whether a reduction in MRI detectable muscle water is a useful surrogate marker for disease progression and treatment response.

Scientific discovery and experimental medicine plans in relation to ryanodine receptor channelopathies (FM, SR, MD): Ryanodine receptor mutations cause disabling core myopathy but the pathophysiology is unknown and there is no treatment[46]. Morphological studies indicate that mitochondria are early targets in the disease but mitochondrial function has not been investigated. We have a funded programme of research to use biobank myotubes to fully evaluate mitochondrial function (see ryanodine channelopathy science programme in appendix II).

Scientific discovery and experimental medicine plans in congenital myasthenic channelopathies (HL, DB, JP FM, SR). Key areas of planned discovery i) New gene discovery next generation whole exome in families not accounted for by known genes. ii)Pathogenic mechanisms; we will establish model systems to study GFAT1 deficiency: patient material (muscle and muscle cells), down regulation of GFAT1 expression in cultured cells by siRNA and zebrafish as a GFAT1 deficient in vivo model; development of appropriate mouse models (in vivo electroporation, knock-out mice) (See appendix II).

D. Mitochondrial Diseases: discovery science and experimental medicine

NCL lead: Doug Turnbull (DT), UCL lead: Michael Hanna (MH).

Background Importance: Mitochondrial myopathies are increasingly recognised as an important cause of muscle disease. Myopathy may be isolated or be part of a multisystem disturbance in which muscle involvement is often prominent and disabling. The clinical severity varies from mild ptosis late in life to neonatal onset severe muscle weakness with respiratory failure. The underlying biochemical defect involves the mitochondrial phosphorylation system. Oxidative phosphorylation uniquely relies upon gene products of the mitochondrial and nuclear genome thus mitochondrial myopathies can be caused by mutations in either genome[54]. Considerable progress has been made in improving the diagnosis and care of patients with mitochondrial myopathies at least in part by establishing the NHS Highly Specialised Services lead by Centre PIs (DT, MH) (UCL, NCL and Oxford) working closely together.

Discovery science and experimental medicine we delivered

Over the last five years we made several major advances including first completed randomised control trial[14], the development of an extensive cohort of patients with accurate genotype and phenotype (MRC funded to UCL and NCL), new approaches to prevent transmission[15] and significant evidence for the benefit of exercise[13]. We have therefore laid the foundations for real translational impact, and the next five years present a major opportunity to translate these advances into clinical practice by developing our experimental medicine programme, by linking with industry and performing informative trials on the stratified cohort. Our MRC support has enabled us to make considerable progress in our understanding and treatment of patients with mitochondrial disease and myopathies as highlighted in recent publications (see CVs). We would like to highlight the following areas:

- We made major insights into the pathogenesis of the mitochondrial myopathy seen in patients with HIV. This work showed that clonal expansion of pre-existing age-related somatic mtDNA mutations and a biochemical defect that can affect up to 10% of cells. These observations add weight to the role of somatic mtDNA mutations in the ageing process and raise the spectre of progressive iatrogenic mitochondrial myopathies emerging over the next decade[55].
- We pioneered new clinically relevant methods to prevent the transmission of mitochondrial myopathies and disease[15]. This work has important policy implications and in response the Secretary of State for Health requested the recent report from the HFEA (http://www.hfea.gov.uk/6372.html). This research was highlighted in the MRC Annual Review as one of the most compelling discoveries of 2010/11 by thinking about medical research challenges from a new angle (http://perspectives.mrc.ac.uk/).

- The MRC funded studies were crucial to a successful bid to MRC/NIHR Translational Medicine Board for funding of the MRC Centre for Translational Research in NMD - Mitochondrial Disease Patient Cohort (UK). This cohort allows careful stratification of patients and helped enable the first ever large-scale randomised controlled trial in patients with mitochondrial disease[14]. There are 12 on-going clinical studies involving patients registered in the cohort from both UCL & NCL.
- An award of Pump-Priming translational research initiative - MRC Muscle Assessment & Training Laboratory. The support from the translational research initiative has enabled us to develop exercise as a therapy for patients with NMD. These ongoing cross-Centre exercise studies have shown that not only is there improvement in strength and oxidative metabolism, but also improvement in quality of life and no adverse effects on mitochondrial function[13]
- Our previous MRC funded work led directly to funding by the NHS Highly Specialised Services Group for a multidisciplinary clinical service for all patients with rare mitochondrial disease in the UK with the centres at UCL, NCL and Oxford. We have a leading international role in the clinical care of patients with mitochondrial disease with the development of assessment scales[56] and guidelines http://www.mitochondrialncg.nhs.uk/newcastle_guidelines.html)
- We have led the first multinational randomised controlled trial in the treatment of a mitochondrial disorder. Licensing negotiations are currently under way with the European Medicines Agency (EMA)[14].

Added value from the MRC Centre core activities: a major added value has been the close working relationship of the MRC Neuromuscular Centre and NCL University Centre for Brain Ageing and Vitality supported by the BBSRC, EPSRC, ESRC and MRC as part of the cross-council Lifelong Health and Wellbeing Initiative (Director Turnbull); and the Newcastle NIHR Biomedical Research Centre (BRC) in Ageing and Chronic Disease (Director Chinnery). Both MRC Centres and the NIHR BRC Centres have a major interest in age-related muscle disease (particularly inclusion body myositis – see appendix II) and sarcopenia, and have worked together on developing exercise programmes and have jointly held workshops exploring the role of exercise as a potential therapy for muscle disease (http://www.cnmd.ac.uk/) A key component of the collaborative activity has been the support of supporting meetings, workshops and both clinical and non-clinical studentships. Finally, the award of MRC Pump-Priming translational research initiative-MRC Muscle Assessment and Training Laboratory was also dependent upon the support of both the MRC Neuromuscular Centre, NIHR BRC and Centre for Brain Ageing and Vitality.

New mitochondrial discovery science and experimental medicine studies

Our proposed studies are dependent upon core areas of support provided by the MRC Centre – the Biobank, MRC Mitochondrial Disease Cohort UK and exercise facilities at NCL and UCL.

Why is muscle so prominently involved in mitochondrial DNA disease? One intriguing aspect of mtDNA disease is the observation that muscle is the most severely or only affected tissue in patients with some mtDNA mutations[57,58]. This observation is particularly intriguing because we know at least some of these mutations are present in the oocyte since low levels of the mutation are present in the monozygotic twin[59]. We will use the clinical data present in the MRC Mitochondrial Disease Cohort and the type and degree of heteroplasmy of individual mtDNA mutations. In addition, we will explore the presence of mutations in muscle satellite cells and other tissues from the patients. Our hypothesis is that those mutations which are lost rapidly from satellite cells are those which should segregate most specifically to post-mitotic muscle.

Why do patients with mitochondrial myopathies get worse? Disease progression is often associated with progressive muscle involvement. Previous studies analysed repeat biopsies from a limited number of patients and suggested the mechanism of progression may depend on the particular mtDNA mutation. In some patients there is increased mtDNA mutation load whereas in others there is decreased mtDNA copy number[57,60,61]. We will re-biopsy patients with different mtDNA mutations in whom we have detailed information on their clinical progression. We will determine the biochemical defect in individual muscle fibres, assess mtDNA mutation load and mtDNA copy number to explore the molecular mechanisms involved in progression for a number of specific mtDNA mutations, determining why these parameters change with time, by correlating the mtDNA changes with the associated respiratory chain deficiency in individual muscle fibres. These studies will provide crucial information on possible therapies since approaches which increase mtDNA copy number (e.g. exercise), could be an effective for some, but not all patients. These investigations will lead to planned intervention studies in stratified patient groups.

What is the response of mitochondrial myopathy patients' muscle to exercise? Several previous studies, including our own, have shown that exercise is beneficial for patients with mitochondrial myopathies[13]. The molecular studies on muscle biopsies have predominantly been limited to exploring

mutation load and copy number whilst there is considerable opportunity to explore the molecular mechanisms which will guide the type of training for individuals. We will use muscle biopsy samples, available in the MRC Biobank, to explore the molecular mechanisms in individual muscle fibres using both new biochemical techniques and RNA profiling[62,63] of muscle fibres. These studies will link directly to the results of the exercise studies available for each individual patient.

Do licensed drugs known to induce mitochondrial biogenesis improve muscle strength and quality of life in mitochondrial myopathy? Emerging evidence from animal models has shown the effect of sirtuins, bezafibrate and rosiglitazone on mitochondrial biogenesis in healthy animals and animals with a tissue specific defect of oxidative phosphorylation. There is limited human data. We will carry out the first placebo-controlled studies of these drugs, evaluating the cellular and biochemical consequences of the drugs, and the clinical consequences of the drugs in terms of muscle and cardiac function, and quality of life. We will also study the way that these treatments interact with exercise. This work will translate some of our earlier studies into clinical practice, and shape the treatment of these disorders in the short-to-medium term future.

Is muscle regeneration a realistic option for patients with mitochondrial myopathies? For a group of patients with mitochondrial myopathies due to sporadic mtDNA disease, the causative mutation is present at high levels in mature muscle, but surprisingly at very low levels or absent in myoblasts from the same patient[59]. Recent studies by MRC funded students have shown that in patients with sporadic large-scale single deletions, the mutation load is similar between satellite cells and mature muscle, but in some patients the mutation is lost rapidly during culture, whilst in others the loss is much slower. However, in all patients the level in replicating cells falls, and this may allow an opportunity for regenerating muscle to lower the level of mutated mtDNA in muscle. We will develop these studies by careful analysis of muscle biopsies from patients who have undertaken resistance exercise training, looking specifically at correlation of the amount of regeneration with mutation load.

4. Training Plans

Education, training and capacity building are major components of the MRC Centre. Our overarching aim is to build a community of translational research students, integrated between both sites and with existing Wellcome and NIHR funded-programmes, and create a self-supporting critical mass of talented future basic and clinical NMD scientists.

Overview: The key strategic training aim was to develop a four-year basic science and a three-year clinical science translational PhD programme to address the severe lack of capacity in the NMD field. We successfully developed and implemented both programmes (details of programmes, recruitment and metrics in appendix III). We delivered the original training strategy and met all objectives.

Recruitment: Eight MRC funded students were recruited to the four-year programme and two students to the clinical science programme (full details in appendix III). Given the huge demand (~forty applicants per post) we sought additional funds to enable more students to enter the programme. We recruited five additional basic science PhD students funded by other sources. For example our strong links with patient organisations enabled some PhD student funding. The training and educational activities of the MRC Centre have been extremely popular and include seminars, web-seminars, workshops and the flagship annual MRC Centre translational science conference (http://www.cnmd.ac.uk/).

People Development and Mentoring: The PhD programmes are jointly delivered between UCL and NCL with a clear focus on translational research. The first four students in the four-year basic science programme have now all submitted their PhDs. The final four students are on target to finish and submit in 2012. The details of the programmes, the supervision/mentoring arrangements and the appointed students and their project titles are given in appendix III. The unique feature of this PhD programme is that during the first six-week induction, the students attend clinics to see patients and had a series of lectures focused on NMD. Following induction, in the first year at UCL, the students rotated three-monthly through three PI laboratories of their choice. Following this rotation the students select their preferred project for their PhD. In the first year in NCL, the students undertake an MRes in Medical and Molecular Biosciences This is a one-year, full-time programme and provides a broad-based training in contemporary molecular biomedical sciences (details appendix III). Following the MRes students commence a three-year PhD project. The two students in the three-year clinical PhD programme undertook either a laboratory or clinical translational (e.g. development of MRI) project together with a significant clinical component (e.g. clinical trial, natural history study). All students are encouraged to attend the multiple scientific educational opportunities in the Centre (developed for all members, senior and junior in the Centre) during their PhD (details; appendix III) including the annual UK Neuromuscular Translational Conference, an annual dedicated neuromuscular clinical update course, monthly MRC

seminar series invited international speakers (also available as web-seminars), regular journal clubs, departmental research meetings and seminars, subject specific workshops and conferences. In addition, the students organised and ran very successful yearly science retreats (http://www.cnmd.ac.uk/). Trainee next destination is in appendix III.

Active Partnering with Stakeholders: the Centre successfully linked with both host universities to strengthen the training programmes. The links include both UCL and NCL students joining a week-long NMD module of the UCL Institute of Neurology MSc in Clinical Neurosciences. In addition, in Newcastle, students successfully joined the MRes in Medical and Molecular Biosciences as the first year of the four-year PhD programme. Strong links between the Centre and the host NHS Trusts enabled the basic science students to attend NMD clinics in the introductory six weeks in year one of the four-year programme. The expansion of the programme to include students funded by other sources e.g. MRC and Wellcome training fellowships, MDC, fellows from the NIH funded RDCRC, has greatly strengthened the training programmes. *Throughout the programme students frequently have opportunities to meet and be inspired by patients and families in order to understand the real impact of NMD on patients' lives.*

Strategic Capacity Building: the lack of capacity of trained UK NMD basic and clinical scientists was the major driver for developing this programme. The MRC funding has enabled us to train four basic science PhD students and one clinical scientist (with five more being trained). We are proud that all five students who completed the programme have continued as postdoctoral or clinical scientists in NMD.

Evaluation and Feedback: very positive feedback indicates we developed a successful and popular PhD programme. We will continually improve the programme as follows:

1) Align PhD programme projects: with the five disease themes, as they have the largest critical mass of world class expertise and the most imminent translational potential.

2) Link MRC Centre PhD students with new training opportunities: that are now available across UCL and NCL. These include a) a new UCL four-year clinical neurosciences modular PhD programme; b) the Wellcome Trust funded PhD programme in Translational Medicine and Therapeutics (Chinnery) in NCL; c) the NCL NIHR Training School developed through the NCL Biomedical Research Centre (Chinnery, BRC Director, Turnbull Theme lead); and c) the Wellcome Centre for Mitochondrial Disease in NCL (Turnbull, Chinnery, Taylor). NCL has developed MRes modules with taught programmes in translational medicine, clinical pharmacology, regenerative medicine, and mitochondrial medicine. Several modules are already delivered by e-learning. We will enable all students across both sites to share all educational opportunities including live web.

3). Build on the success of the Wellcome programme in Translational Medicine and Therapeutics. We will consolidate links with industry through industrial placements and industry-shared training programmes. The NCL MRes module on therapeutics includes webcasts by industrial partners, which will be available to all UCL and NCL students. GSK have agreed to fund two CASE PhD studentships in MRI studies (see MRI theme in appendix II).

4). One year post CCST translational fellowships. There is a need to offer one-year translational fellowships to senior clinical trainees e.g. post-CCST. This is particularly important for trainees wishing to pursue a clinical translational NMD career (e.g. in clinical trials) and who will contribute to future UK trials networks. MR leads a European TREAT-NMD group that developed an NMD advanced fellowship now offered in the Centre (appendix III). We are not requesting MRC funding for this programme, but these trainees do benefit significantly from the Centre's educational and translational environment.

Future Plans: We intend to increase the capacity of our PhD programmes. We request MRC funding for nine PhD students to enter the four-year programme and have agreement for 1-1 host matched funding. GSK have agreed to provide two CASE PhD studentships for MRI development. We request MRC funding for two three-year clinical PhD students (one UCL, one NCL), also host-matched. In order for more clinician scientists to be trained in translational medicine we devised a brand new one year clinical science "pump priming" programme to allow very talented clinical trainees one year to obtain pilot data before applying for MRC training fellowships. We request four MRC "pump-prime" fellowships which will be host-matched.

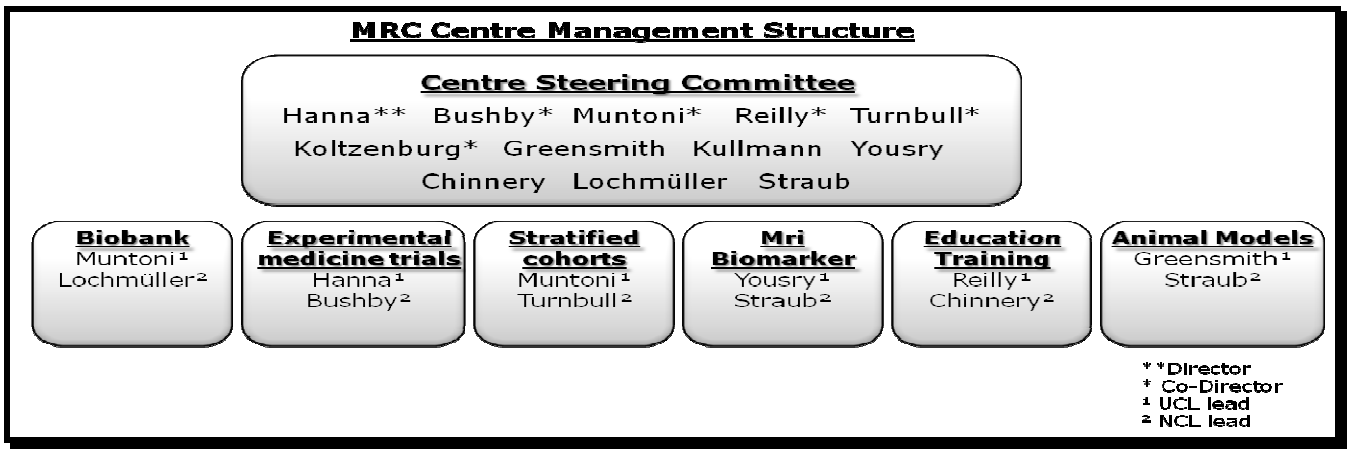
5. Institutional commitment

Host institution commitment letters from UCL Provost and NCL Vice Chancellor attached as appendix I.

6. Management

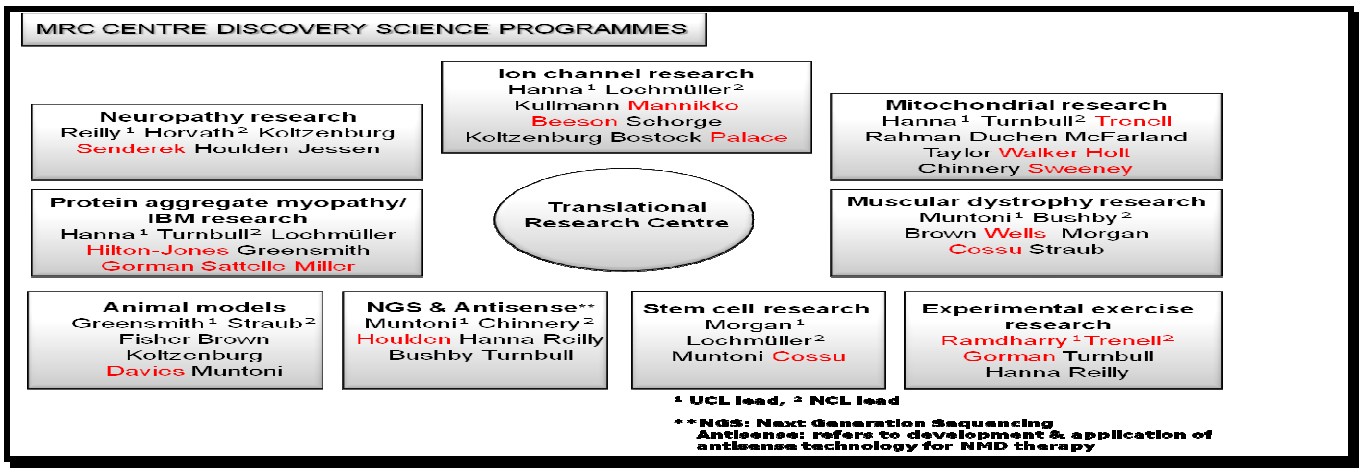
The Director had full institutional support and direct access to senior colleagues in both universities. At UCL, MH meets regularly with Alan Thompson, Dean of the Faculty of Brain Sciences. In NCL, KB & DT meet regularly with Chris Day, Pro-Vice Chancellor & Provost of Medical Sciences. This strong support

and clear alignment with host mission is reflected by the substantial 1-1 matching support (>£3.6m direct & indirect costs). We developed an effective and collegiate Centre management structure. This structure ensures delivery of key management functions: **operational, oversight, involvement, scientific** & **strategic**. The Director chairs a monthly steering committee in which UCL & NCL colleagues consider an agenda including standing items on each core translational activity. All meetings are minuted, are face to face whenever possible, or teleconferences. This format has proved an effective and productive mechanism to ensure efficient **operational** Centre running; ensures peer colleague **oversight** including allocation of resources and enables **strategic** planning. A particular strength of the NCL-UCL team is our extensive global reach (e.g. Turnbull: European & USA mitochondrial initiatives; Bushby-Straub-Muntoni: TREAT-NMD European network of excellence; Hanna: NIH consortium for channelopathies, North American Muscle Study Group scientific member, chairman British Myology Society, NIH NeuroNEXT links; Reilly: NIH genetic neuropathy consortium) ensuring an international dimension to strategy & regarding opportunity and influence. **Public/patient/science community involvement**: the steering committee successfully delivered the largest UK annual NMD translational research conference with the biggest UK patient organisation & Centre project partner–the Muscular Dystrophy Campaign and a series of successful patient & patient organisation days (http://www.cnmd.ac.uk/). The four annual meetings attracted >1200 delegates and rotated between UCL, NCL, Oxford. To ensure rigorous independent **scientific** review we established an international SAB including some of the most highly respected NMD world experts. The SAB visited the Centre three times to review science & translational delivery (see two detailed SAB reports appendix III & http://www.cnmd.ac.uk/). In the renewed Centre, the Director (Hanna) will work closely with five senior Co-Directors (UCL Co-Directors: Muntoni, Reilly, Koltzenburg, NCL Co-Directors: Bushby, Turnbull). See simple management diagram below:



7. Overview of Centre scientists contributing to the Centre's key research themes

In addition to previously attracting world class scientists (Muntoni, Morgan, Hughes, Lochmüller), two new eminent scientists will join the Centre in renewal: Giulio Cossu, muscle stem cell expert at UCL and Jan Senderek, a neuropathy expert at NCL. The Centre has critical mass of scientific and translational expertise in each disease-themed area. We have expertise in each new therapy theme (antisense, stem cells, exercise). In addition, new UCL, Kings, Newcastle, Manchester, Oxford and Cambridge collaborators have joined. Diagram illustrates the scientists that contribute to each of the disease themes. Investigators in red have recently joined. Centre PI CV's & science theme details in appendix II.



8. Evidence of outputs and outcomes

The MRC Centre has met the agreed objectives/metrics in relation to each of the core areas (see full pdf at http://www.cnmd.ac.uk/)**: 1.Neuromuscular trials:** we delivered a marked increase in natural history studies and experimental trials from n=3 to n>30. We tested new experimental therapies in man: antisense in DMD, heat shock protein upregulation in IBM, exercise therapy and idebenone in mitochondrial disease, mexilitine in muscle channelopathies & vitamin C in CMT1A, & published in high impact journals e.g.: Brain, Neurology, Lancet, Lancet Neurology[1,3,10,12,13,14]. Patients in trials of new therapies has risen from n=29 to n>200 & we recruited >2000 patients into stratified cohorts. MRC experimental trials coordinators were crucial in supporting these significant increases & ensuring our very high success in recruiting cf other centres world-wide (see SAB comments appendix). **2.Neuromuscular biobank**: we established a national MRC biobank of nerve and muscle tissues available to scientists which has added value to discovery science and preclinical testing of therapies (e.g. antisense in DMD). We far exceeded the original sample target (target 550 samples; achieved >1870). We provided cell lines to 53 different scientists (20 within the MRC Centre; also Europe, Japan, Australia). We have cell lines on >60 different NMD. 22 peer reviewed publications have arisen directly from biobank tissue research, and many others have used the biobank (see full publications appendix II). 5 MRC PhD student projects used the biobank (appendix II). **3.MRI Biomarker studies:**. Development of NMD MRI biomarkers was a completely new initiative established by the MRC Centre. We applied qualitative MRI and developed quantitative MRI methodology in muscle/nerve: ***eight methodology techniques delivered and five new MRI patient studies delivered*** (listed in detail in section 2 above). **4.Animal studies**: we delivered on areas agreed. We established a comprehensive animal phenotying facility that includes behavioural, histological and electrophysiological techniques. We developed novel electrophysiological assessment techniques including nerve excitability profiling and cardiac & skeletal muscle MRI. MRC investigators & PhD students used this facility. 19 peer reviewed publications in which the facility was utilised (appendix II) **5.Training and education**: all objectives/metrics delivered (appendix III). Established four-year translational research PhD programme. Successfully recruited all ten students (>40 applicants for each post). First five have submitted PhD's. **Partnerships industry**: we formed extensive industry experimental medicine partnerships to (GSK, Prosensa, Shire, Senxis, PTC, AVI). **Partnerships patient organisations**: we linked with the Muscular Dystrophy Campaign to establish the largest UK NMD translational research conference-now held four conferences >1200 delegates total. **Partnerships academic**; new links with Oxford (D.Hilton-Jones, K.Davies, M.Wood), Cambridge MRC MBU (J.Walker, I.Holt), **Public engagement:** held six Centre patient days for scientists to link with patients and families; two patient organisation days to enable links with Centre. **PI's publications:** we published > 500 peer reviewed publications 138 include more than one Centre PI. Centre PI's attract >£60m grant funding ~£14m from MRC. **Reputation:** MRC Centre has established an international reputation for translational experimental NMD research with global links (see SAB comments *"arguably the leading Centre in the world for experimental NMD"*). **Developing resources**: we developed a biobank, an animal phenotyping facility, a translational PhD programme, national visible leadership, a network of clinicians for trials (British Myology Society) and national stratified cohorts for personalised medicine and a NMD MRI platform. **Technologies**: we have systematically applied MRI and developed it as a biomarker and outcome measure in NMD-NIH now using our protocol. The proposed SMART metrics for the next funding period are listed in table below:

	Metric	Baseline	Target	How Outputs Measured	Qualitative outcomes
	Resource generation				
1	Numbers of samples biobanked and distributed	1800	3000	Six monthly reporting of metrics and publication in annual report	High impact publications based on work utilising biobank samples
2	Numbers of patients enrolled in NMD cohorts	2000	3000	Six monthly reporting of metrics and publication in annual report	Patient availability for studies, greater patient engagement

3	Validated MR clinical endpoints for trials	8	16	Numbers of patients enrolled in MR studies of clinical outcomes annually	Publications, SOPs for MR evaluation, adoption as trial outcomes
4	Numbers of students enrolled in MRC training programme	9	18	Annual returns, time to PhD completion	Availability of highly trained cohort of scientists
5	Attendees at MRC conference, workshops and seminars	250pa	300pa	Numbers of participants, participant feedback, web hits on podcasts	Greater engagement of NMD clinical and academic community
6	Numbers of additional centres contributing to biobanks, cohorts, trials	0	2	Numbers of samples and patients reported six monthly and annual report	Greater engagement of NMD clinicians, patients
7	New high level science recruitment to MRC centre	4	6	Numbers of new staff attracted to MRC Centre	Greater critical mass
8	Number of industry partnerships	5	10	Numbers of industry contacts and contracts	Greater industry involvement in NMD
	Know how				
9	Number of experimental medicine studies initiated	16	20	Numbers of orphan drugs associated to centres, trials initiated	Proof of principle studies completed, high impact publications
10	Number of new genes identified and classified	6	24	Numbers of new disease genes collected annually	Publications, new disease targets for therapies
11	New targets for antisense therapy in NMD	1	2	Numbers of experimental antisense studies annually	Grants, publications, new clinical studies
12	Initiation of a clinical study for stem cell therapy in NMD	0	1	Milestones to clinical study including safety, preclinical evaluation	Proof of principle clinical study, grants, publications
13	Numbers of experimental exercise studies initiated	1	4	Numbers of patients enrolled in exercise studies annually	Understanding the role of exercise in disease and therapy
14	**Overall scientific/ academic output** **More than one PI**	135	200	Numbers of joint papers and value of grants	Increased high impact publications

	Total PI NMD output	500	600	including MRC, EU	and grant awards

9. Communications

We delivered highly visible strategic leadership and our communication plan engaged all stakeholders including: patients/patient organisations, funders, host Universities, host NHS institutions, host biomedical research centres, UK NMD clinicians and scientists, international colleagues/funders and UK and EU policy makers. **Patients**: investigators in NCL and UCL have a strong record of seeking and incorporating patient and carer opinions through the MRC Centre patient and patient organisation days held regularly (http://www.cnmd.ac.uk/). **Funders/scientists/clinician/charities**: we have strong visible links with major UK charities including the Muscular Dystrophy Campaign (MDC)-a project partner. We established the largest UK neuromuscular translational research science conference in partnership with the MDC. There have been four very successful conferences rotating nationally (UCL, NCL, Oxford) attended by 250-300 scientific delegates. **National clinician networks**; we established the British Myology Society; a UK network of NMD clinicians linked to trials and cohort building (http://www.cnmd.ac.uk/). **Policy makers**: we achieved the first NICE accreditation for NMD (DMD) and highlighted standards of care are improved within experimental trials. We are fully engaged with NHS commissioners and lead nationally commissioned NMD services and have direct links into EU policy making (KB). We link with UCL and MRC media offices as appropriate. (see http://www.bbc.co.uk/news/health-16004112) and (www.mitochondrialncg.nhs.uk/newcastle_index.html).

10. Exploitation

Our discoveries may include new mechanisms/genes/molecules, new diagnostics and new treatments and could create IP issues. Advice from UCL and NCL IP expertise will be sought early. UCL Business PLC manages the IP portfolio of UCL and provides a senior business manager (Chris Loryman c.loryman@uclb.com) to cover the commercial activities with an additional legal/commercial support team. In NCL there are specific links with the university commercial enterprise team and the senior manager contact for the MRC Centre is Martin Cox (martin.cox@ncl.ac.uk).

11. Ethics

All patient-related research will be done in full accordance with REC approval & in line with NCL & UCL R&D governance. Data Protection Preservation for sharing will be done according to MRC and OECD (www.oecd.org). All research/education is within clear governance framework of host universities/NHS organisations. All animal activities are undertaken in accordance with Home Office regulations. Ethical and clinical governance issues relating to biobank comply with UK and EU regulations. This Biobank is already linked to Eurobiobank: (http://www.eurobiobank.org/en/information/info_institut.htm).

12. Resources

The Centre mission is fully aligned with the experimental and translational mission of the host universities. Substantial new grant success, patient impact and publication output metrics of the Centre enabled us to make a strong case for host 1-1 matching with the MRC support requested. We request £3.3m from the MRC which will be more than matched by brand new host support totalling £3.6m, plus recruitment of Giulio Cossu to UCL and Jan Senderek to NCL. **Justification for MRC resources requested:** resources requested are specifically targeted to support and accelerate the core translational activities of the Centre which underpin the two over-riding Centre deliverables: ***1. Increased scientific "know-how" in the area of NMD translational research and in particular the delivery of experimental medicine studies and new gene discovery, and 2. Resource generation to extend the tools which enhance UK competitiveness in this field by the embedding of a trial culture for UK NMD patients.*** In renewal we are not requesting specific MRC support for animal model work which will be supported by other funded programmes of PI research. We established a successful core phenotyping facility with previous MRC Centre support which will continue to add value to Centre investigators work linked to MRC Harwell and Neuromouse consortium. We request support for innovative new workshops to encourage "cross-talk" between animal and clinical NMD scientists which we believe will add new value to existing MRC-animal work investments. **Core activities 1 & 2**: ***Experimental medicine stratified cohorts and experimental trial co-ordination.*** Two experimental medicine coordinator posts are requested from the MRC and these will be matched by host support. Each sister-site of the Centre will have two staff to ensure maintenance and development of the stratified cohorts and to ensure rapid trial design, protocol development and initiation and delivery of complex

experimental trials in children and adults. It is important to note that the rigour which stratified cohorts and experimental medicine studies require for regulatory approval and ongoing assessment to ensure compliance with regulations, and scientific excellence is high. Such studies cannot be initiated and completed without full-time dedicated support over the next five-year period. The work underpinning any given study may take months to deliver and ongoing vigilance and reporting requirements are also major complex tasks. Given the specific complexity of the studies in relation to first in man delivery and data quality for the cohort collections, dedicated support is essential in this area. These posts for five years each are critical for delivering the central mission of the Centre. Such important underpinning posts are not eligible for funding from other external sources. We have used these posts extremely effectively to increase experimental studies from 3 to >30 in just four years. In addition, we have maximally leveraged any NIHR host support e.g. in relation to NHS support costs for certain trials once the trials coordinators have completed all the administrative work to achieve NIHR portfolio status. However, the focus of NIHR is not experimental medicine studies in rarer diseases. The outcomes described under metrics 2, 3, 8, 9, 10, 11 & 13 depend on this MRC investment. **Core activity 3**: ***Biobank support***. In the previous MRC Centre grant the UK NMD biobank was established and sample collection and distribution far exceeded projections, underpinning many high profile scientific publications and grant awards. In renewal we are extending the scope of the biobank towards generation of iPS cells and collection and distribution of samples to increased numbers of centres (metric 6). The biobank underpins a range of activities relating to metrics 1, 6, 9, 10, 11 and 12 and is also attracts industry links (metric 8). We request two biobank positions from the MRC matched by the hosts. This will provide two posts each at UCL and NCL to support existing burgeoning core activities and new developments (section 2 above). **Core activity 4**: ***MRI Biomarker support.*** The Centre is leading internationally in the generation of NMD MRI outcomes and the physics expertise underpinning this is essential to deliver metrics 3, 9 & 13. There is a real prospect that Centre-developed quantitative MRI sequences will become the standard outcome in NMD experimental medicine studies and we can consolidate an international leadership position. We request one physicist from the MRC matched by host support. MRI scan-time requested from MRC has been minimised. **Core activity 5. *PhD students and training programmes***. The Centre PhD programme has been highly regarded and has generated new skilled scientists with 100% completion rates. We had >40 applicants for every post. We want to expand student numbers working in key scientific areas of the Centre, advance scientific "know-how" and provide the next generation of scientists. The PhD students through their work, enthusiasm and dynamism contribute to all deliverable metrics directly or indirectly. We have agreement for 1-1 matching from the host which will enable us to train a total of eighteen four-year non-clinical PhD students. It is extremely important the Centre trains the future clinical translational scientists so we request two three-year clinical PhD students. In addition, we request four one-year "pump-prime" translational clinical fellowships which will allow us to train excellent applicants to obtain pilot data for a year before applying for MRC training fellowships; these will also be host matched. ***Director, PI support and administrative support.*** Support for 20% of the Director's time has been requested, matched by the host, allowing MH to devote at least 40% of his time to the Centre. In NCL 5% of the time of Co-Director KB has been requested, matched by the host, reflecting the importance of the successful UCL-NCL link. Support and co-ordination of the work of the Centre by the Co-Directors is a significant role (DT, FM, MK, MR); the host is providing the other Co-Director investigator costs to deliver these tasks ensuring the Centre once again exceeds its goals and targets. The administrative support for the Centre, although pivotal for the Centre's success, is reduced to 50% from the MRC with 50% from the host.

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