CALENDAR YEAR(s): 2015,2016,2017,2018

AUSTRALIA

Murdoch - Murdoch University

Steve D Wilton Ph.D

RG Oligomer design & validation for DMD: quantum improvements in exon skipping

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Unequivocal dystrophin expression has been demonstrated in DMD boys receiving a morpholino oligomer designed to restore the reading frame of their DMD gene transcripts. Results from on-going clinical trials indicate that the level of dystrophin expression is now being translated into tangible benefits. Challenges include establishing the most effective dosage regimen, and implementing trials to address the spectrum of dystrophin mutations most amenable to targeted exon skipping. Exons within the major deletion hotspot are regarded as high priority targets as their removal would be relevant to many DMD individuals. We seek to develop more efficient exon skipping compounds, not only for the high priority targets but also other exons, particularly in the rod domain, whose omission from the dystrophin mRNA will allow synthesis of a functional dystrophin isoform. We have identified several pathways to enhance splice switching efficiency, including retrospective bioinformatic analyses of effective and ineffective oligomers, evaluating oligomer combinations, novel features in oligomer design (mismatches) and targeting exonic domains not previously tested. Design of the most efficient splice switching oligomers for DMD will ensure the best clinical outcomes and extend the treatment to other DMD mutations.

Parkville - The University of Melbourne

Gordon Stuart Lynch Ph.D.

RG Therapeutic potential of heat shock protein 72 induction in muscular dystrophy

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Muscle wasting and weakness are major symptoms of many neuromuscular disorders, including Duchenne muscular dystrophy (DMD). Although considerable efforts are being directed to the development of gene therapies for DMD, these techniques are far from perfected. In the interim, it is essential that alternative therapies be developed, and research directed to preserving muscle tissue, enhancing muscle regeneration, and promoting muscle growth. Through MDA support, we have made major contributions to the field; demonstrating that growth factors have exciting potential for improving muscle function in the mdx mouse, an animal model for DMD (Am. J. Pathol. 161:2263-72, 2002; Muscle Nerve 30:295-304, 2004; Am. J. Pathol. 166:1131-1141, 2005; Exp. Physiol. 93:1190-8, 2008; Am. J. Physiol. 294:C161-8, 2008; and many other published papers and review articles). We have recently discovered how Hsp72 induction (through transgenic manipulation, heat therapy and drug-induction) can protect dystrophic muscle against functional decline and improve lifespan in severely affected dko mice (Nature, 484, 394-398, 2012). Based on this novel and important biological discovery, this research proposal aims to examine the full therapeutic potential of Hsp72 induction in the skeletal and cardiac muscles of various models of muscular dystrophy, with the aim of developing a novel treatment for improving skeletal and cardiac muscle function and quality of life for patients with muscular dystrophy.

Sydney - The University of Sydney

Joshua Burns Ph.D

HCTG Strength training for children with Charcot-Marie-Tooth disease: Help or Harm?

$142,419.00	3/1/2014	2/28/2015	Year 3
$150,634.00	3/1/2015	2/28/2016	Year 4

Summary Charcot-Marie-Tooth disease (CMT) is the most common neuromuscular disorder. The most debilitating problem for people with CMT is weakness. There is no cure. Progressive resistance strength training has the potential for benefit, but equally it may cause harm. Our pilot data show a reversal of weakness and improved function. We will conduct a 2-year randomized double-blind, sham-exercise controlled trial to investigate the efficacy and safety of progressive resistance strength training in CMT.

BELGIUM

Gent - VIB vzw

Ludo Van Den Bosch Ph.D.

RG Role of HDAC6 in Charcot-Marie-Tooth disease.

$78,340.00	5/1/2014	4/30/2015	Year 1
$78,340.00	5/1/2015	4/30/2016	Year 2
$78,340.00	5/1/2016	4/30/2017	Year 3

Summary Based on mutations in the HSPB1 gene, one of the genetic causes of Charcot-Marie-Tooth disease and distal Hereditary Motor Neuropathies (distal HMN), we have created transgenic mouse models for both diseases. These transgenic mice show similar signs as the patients and we can cure the CMT2 mouse model by a treatment with a selective histone deacetylase 6 (HDAC6) inhibitor. HDAC6 is the major tubulin deacetylating enzymes present in peripheral nerves and it plays an important role in the regulation of axonal transport. In this project, we will investigate the exact mechanism responsible for the mutant HSPB1 induced axonopathy and we want to obtain a better understanding of the therapeutic effect induced by inhibition of HDAC6 In addition, we will also investigate the therapeutic potential of HDAC6 inhibitors by treating other animal models of CMT and distal HMN.

CANADA

NOVA SCOTIA

Halifax - AGADA Biosciences

Kitipong Uaesoontrachoon PhD

RIG Murine Preclinical Center for Neuromuscular Diseases (MPCNMD)

$100,000.00	3/15/2014	3/14/2015	Year 3

Summary Recent advances in high throughput drug screening are facilitating identification of several drug candidates for muscular dystrophy. Currently there are very few murine preclinical facilities that can screen these therapeutic candidates in a reliable and reproducible manner in mouse models of neuromuscular diseases. The preclinical drug testing facility at Children's National Medical Center (CNMC) is one of the few facilities in the US that is equipped with state-of-the art equipment to comprehensively assess therapeutic efficacy of drugs/ compounds in multiple models of myopathy in a robust and reliable manner. The Murine Preclinical Center for Neuromuscular Diseases (MPCNMD) at CNMC will use standardized protocols for skeletal, respiratory and cardiac endpoints in mouse models that will help to guide planning human clinical trials. The MPCNMD will maintain rare models of muscular dystrophy; develop new methodologies for phenotyping and screen therapeutics coming from both academic and industry groups. It will serve as a premier pre-clinical core facility for muscular dystrophies so that patients will have access to the best potential therapeutics as quickly as possible.

ONTARIO

Hamilton - McMaster University

Vladimir Ljubicic Ph.D.

DG Dissecting the mechanisms underlying the benefits of novel therapeutics for DMD

$60,000.00	2/1/2014	7/31/2015	Year 3

Summary A strategy to counteract Duchenne Muscular Dystrophy (DMD) consists in utilizing a protein normally expressed in dystrophic muscle that, once expressed at appropriate levels and at the correct subcellular location, could functionally compensate for the lack of dystrophin. A candidate for such a role is utrophin. Muscle fibers from DMD patients express utrophin endogenously. Some muscle fibers (i.e., “slow-twitch” fibers) express utrophin to a greater extent than others (i.e., “fast-twitch” fibers), and these muscles display an elevated level of protection from the disease. Induction of slow-twitch fibers, which includes utrophin upregulation, bears functional improvements for dystrophic muscle. However, a critical question is whether the beneficial adaptations induced by evoking the expression of slow fibers in dystrophic muscle is strictly dependent on the upregulation of utrophin or on one of the other changes affiliated with the slow-twitch phenotype. My research plan appears particularly timely and important since several compounds that will be employed in my investigations to elicit the expression of slow-twitch fibers are currently being evaluated in clinical trials for a variety of metabolic diseases. This could therefore greatly accelerate the development and implementation of novel therapies for DMD centered on utrophin upregulation and/or promotion of the slow-twitch phenotype.

Ottawa - Ottawa Hospital Research Institute

Rashmi Kothary Ph.D.

RG Modulating actin dynamics as a therapeutic strategy for spinal muscular atrophy

$84,600.00	5/1/2014	4/30/2015	Year 1

$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary A pathological hallmark of spinal muscular atrophy (SMA) is the loss of lower motor neurons in the spinal cord and corresponding muscular atrophy with subsequent paralysis and in most severe cases, death of young babies. Mutations in the survival motor neuron 1 (SMN1) gene are causative of SMA. To date there is no cure or effective treatment for SMA, and most interventions are designed to simply improve disease symptoms. With previous MDA funding, we demonstrated that administration of inhibitors of the RhoA pathway, namely the Rho kinase (ROCK) inhibitors Y-27632 and fasudil, leads to a dramatic increase in survival in a mouse model of intermediate SMA, concurrent with improvement in integrity of neuromuscular junction and increase in muscle fiber size. Furthermore, this benefit to the SMA mice was SMN-independent. These studies identified RhoA effectors as viable targets for therapeutic intervention in the disease. As both fasudil and Y-27632 are relatively weak inhibitors, additional inhibitors with novel structures and improved potency and selectivity may provide better tools to further evaluate the therapeutic effect of ROCK inhibition on various aspects that contribute to the pathogenesis of SMA. Our objective is to identify a development candidate with potent inhibitory activity at ROCK, highly brain permeable and a favorable safety profile. In this proposal, we will test ROCK inhibitors currently under development at Theratrophix.

Lynn Megeney Ph.D.

RG Caspase 3 Limits the Renewal of Activated Satellite Cells

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Growth and repair in post natal skeletal muscle is controlled by a stem cell population referred to as satellite cells. As such, satellite cells are the ideal source for repairing or replacing damaged skeletal muscle that is associated with injury or disease. Although the mechanisms that regulate the final steps of satellite cell maturation into muscle fibers are well understood, we have little understanding of what controls the behavior of these cells at earlier stages, i.e. what factors renew these stem cells and what factors initiate the first steps in the maturation process. Here we are investigating the role of the caspase 3 protein in satellite cell behavior. We have shown that caspase 3 limits the ability of satellite cells to remain as stem cells and encourages the key first step in the maturation process to muscle. We propose to investigate the mechanisms by which caspase 3 controls this vital cell decision.

Ottawa - University of Ottawa

Bernard Jasmin PhD

RG The RNA-binding protein Staufen1 as a target for novel therapies for DM1

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Myotonic dystrophy type 1 (DM1) is caused by mutations in the DMPK gene. The presence of this mutation is thought to lead to aberrant patterns of interactions between proteins normally expressed in cells and mutant DMPK messenger RNAs. In turn, these aberrant interactions prevent these proteins from assuming their normal functions within DM1 cells thereby causing many symptoms characteristic of this disease. In this project we will examine the role of one such protein, Staufen1, which interacts with DMPK messenger RNAs and whose expression and localization is markedly affected in DM1 muscle. The identification of such proteins and the elucidation of their functions in skeletal muscle are important since these studies may lead to the development of new therapeutic strategies for treating DM1.

QUÉBEC

Montreal - Centre de Recherche du Centre hospitalier de l'Universite de Montreal

Alex Parker Ph.D

RG Investigating the ER stress response in TDP-43/FUS motor neuron toxicity

$75,100.00	8/1/2014	7/31/2015	Year 3

Summary TDP-43 is a recently identified gene associated with Amyotrophic Lateral Sclerosis (ALS). Very little is known about how mutations in TDP-43 cause ALS. My goal is to better understand both the normal biological role of TDP-43 as well as the mechanism of neuronal toxicity in the pathological condition. For neurodegenerative diseases, the path from genetic mutation to neuronal dysfunction and cell death is complex and in humans takes many decades. Thus, convenient systems are needed to pursue the manipulation of neuronal survival at a comprehensive, genome and organism-wide level. Using invertebrates like C. elegans, to model human disorders has emerged as a useful strategy in the neurodegeneration field. I will use worm deletion mutants

of TDP-43 to learn more about the gene's normal biological roles in the cellular stress response. This information may shed light on disease mechanisms in ALS patients. I have also created transgenic worms that express mutant human TDP-43 in motor neurons that I will use to discover genetic mechanisms to reduce neurodegeneration. My preliminary data suggests a role for the unfolded protein response in TDP-43 & FUS motor neuron toxicity. Further understanding the role of TDP-43 in stress response signaling may aid drug discovery efforts to arrest disease progression and provide a better quality of life for ALS patients.

Montreal - CHUM Research Center

Christine Vande Velde Ph.D.

RG Impact of TDP-43 on stress granule signaling in ALS

$119,414.00	8/1/2014	7/31/2015	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of specialized neurons that control voluntary movement. The mechanism and the biological basis of specificity of how these specialized neurons, known as motor neurons, are lost in ALS remains unknown. A combination of internal stress (genetic mutation) and external stress (environmental factors) are believed to contribute to ALS pathogenesis. A variety of environmental influences have long been linked to ALS, and motor neurons are known to be very sensitive to chemicals and environmental stress. Mammalian cells possess a variety of mechanisms to mediate a cell's recovery from physiological and environmental stresses. We have recently identified TAR DNA binding protein (TDP-43) as a regulator of one cellular stress response: the formation of stress granules. TDP-43 is well described as a causative gene for ALS. Thus, this project is aimed at exploring the biochemical signaling in stress granule dynamics mediated by TDP-43 and understanding how disease-causing mutations may disrupt these processes.

Montreal - McGill University

Heather D. Durham Ph.D.

RG Altered trafficking of FUS in motor neurons and relevance to ALS

$118,611.00	2/1/2014	1/31/2015	Year 2
$118,003.00	2/1/2015	1/31/2016	Year 3

Summary Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease) is a fatal neurodegenerative disorder characterized by the loss of motor neurons that relay messages from the brain to skeletal muscles. The result is gradual loss of the ability to move, to swallow, to speak and eventually to breathe, but most other bodily functions remain intact. Although the cause of most cases of ALS is not known, genetic mutations have been identified in forms that run in families. One such form, ALS6, is caused by mutations in FUS, a constituent of protein complexes that transport RNA from the nucleus throughout the cell where it serves as the template for synthesis of proteins where they are needed. In neurons, the distribution of these RNA-containing complexes is particularly important for maintaining synaptic connections with other neurons and for responding to the level of neuronal activity and stress. In ALS6, as well as in sporadic disease, the distribution of FUS in motor neurons can be abnormal. Our research will determine how mutations affect trafficking of FUS and its partners including RNA, how these abnormalities relate to and/or affect cellular adaptive responses, and if the microenvironment created in other forms of ALS could disrupt the function of normal FUS. The latter speaks to how chronic stress affects the function of motor neurons.

Montréal - Institut de recherches cliniques de Montréal (Clinical Research Institute of Montreal)

Benoit Coulombe Ph.D.

RG Regulation of the inclusion body myositis-associated protein VCP by methylation

$125,689.00	2/1/2014	1/31/2015	Year 2
$125,689.00	2/1/2015	1/31/2016	Year 3

Summary Tissue degeneration is a hallmark of many muscular and neurological diseases. In many cases, such as inclusion body myositis (IBM), myofiber degeneration was proposed to be due to an abnormal accumulation of toxic oligomers of misfolded proteins within affected cells. Normally, these aggregates are eliminated through specific cellular mechanisms and machineries. Identifying genes and proteins either involved in the accumulation of protein aggregates or able to prevent such a process is paramount to our understanding of these illnesses and represent candidate targets for biomarker and drug discovery. One such protein, the Valosin Containing Protein (VCP), is involved in normal processing of misfolded proteins, and mutations in its gene are often the cause of a subset of familial IBM and Amyotrophic Lateral Sclerosis (ALS). Recently, our group identified a novel enzyme that specifically modifies VCP and regulates its ATPase activity, raising the possibility that it could regulate its role in abnormally folded protein degradation. In this program, we will study the regulation of VCP by this novel enzyme, particularly when VCP is affected by IBM and ALS causing mutations. The interest of this study is to (i) discover new tools for modulating VCP activity or even preventing its impairment in IBM and related diseases, and (ii) develop specific and sensitive assays that use

VCP and its interactors as biomarkers to screen for various degenerative diseases.

CYPRUS

Nicosia - CING - The Cyprus Institute of Neurology and Genetics

Kleopas A. Kleopa M.D.

RG Developing gene therapy for inherited neuropathy

$93,287.00	8/1/2014	7/31/2015	Year 2
$95,995.00	8/1/2015	7/31/2016	Year 3

Summary Our aim is to develop and test a novel gene therapy for a common inherited neuropathy, the X-linked form of Charcot-Marie-Tooth Disease (CMT1X). CMT1X is caused by mutations affecting the gap junction protein connexin32 (Cx32). Cx32 forms connecting channels between layers of the myelin sheath and plays an important role in peripheral nerve function and integrity. Patients with CMT1X develop slowly progressive muscle atrophy, weakness and sensory loss in the limbs. There is no effective treatment for CMT1X. We have generated mouse models of CMT1X expressing human mutations and showed that the mutations cause loss of Cx32 function and progressive neuropathy, similar to mice lacking the Cx32 gene. Therefore, gene replacement may be a promising future therapeutic approach. We have already engineered and produced special viral vectors able to deliver and express the Cx32 gene in peripheral nerves and have demonstrated that direct delivery of these vectors to the sciatic nerve of mice results in sustained and widespread production of the protein. Based on these encouraging results, we propose to study a combination of gene delivery methods to reach peripheral nerves, including direct injection into the nerves, muscles, and the lumbar root area. We will then treat mice lacking the Cx32 gene and examine clinical, physiological, and pathological effects of the treatment. Finally we want to prove that even in mice expressing human Cx32 mutations this therapy could still be effective.

FRANCE

Illkirch - CERBM GIE

Jocelyn Laporte Ph.D. Molecular Biology

RG Genetics and Physiopathology of Tubular Aggregate Myopathies

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Tubular aggregate myopathies (TAM) are characterized by progressive muscle weakness affecting the lower limbs and associated with muscle pain and cramps. On muscle biopsies, TAM show regular arrays of membrane tubules in muscle fibers. These aggregates can also be found as secondary features in various muscle disorders and accumulate in normal muscle with age. We recently identified a first gene implicated in primary TAM and encoding for a calcium sensor. Calcium triggers muscle contraction and is a key molecule for muscle growth and differentiation. Consequently, the intracellular calcium flow has to be tightly regulated to ensure normal muscle function. We demonstrated that the identified mutations impact on the calcium level in muscle cells, but the exact disease mechanisms leading to muscle dysfunction and pain remain unknown. Therefore, we will analyze the nature, the origin and the impact of the tubular aggregates and the correlated calcium defects in cells and in an animal model. Moreover, we will test a potential therapeutic rescue using selected drugs acting on the calcium flow in both patient cells and animal model. As several patients of our TAM cohort do not harbor mutations in the previous gene, we will finally identify further TAM genes, potentially representing novel drug targets. This project will contribute to a better understanding of several myopathies and muscle aging.

GERMANY

Berlin - Freie Universität Berlin

Peter Robin Hiesinger Ph.D.

RG A Drosophila Model for Charcot-Marie-Tooth 2B Disease

$50,000.00	8/1/2014	7/31/2015	Year 2
$50,000.00	8/1/2015	7/31/2016	Year 3

Summary Charcot-Marie-Tooth Disease type 2B (CMT 2B) is a sensory neuropathy caused by mutations in the gene rab7. This gene encodes a protein with a critical function in the degradation of intracellular debris in all cells. In patients, weakening and ‘dying back’ neuromuscular contacts in early adulthood lead to neuropathy symptoms, including severe sensory loss in limbs. Even though the gene is known, it is unclear how the

mutations found in patients affect the gene's function. The disease is dominant, i.e. one mutant copy and one normal version of the gene in patients are sufficient to cause the disease. It has therefore been proposed that the CMT 2B-causing mutations lead to increased function. We propose that this currently assumed reason for the dominance is incorrect. We have established the first animal model for CMT 2B using Drosophila as a model animal and primary rat neuronal culture for validation experiments. Our preliminary data show that dosage-dependent loss of rab7 gene function affects nerve cells before other cells in the body. Our findings explain the genetic dominance and reveal a particular sensitivity of nerve cells for a defect in debris removal. This discovery opens the door for an understanding and a potential therapy of CMT 2B based on the molecular manipulation of the underlying cause. Importantly, we suggest an increase of rab7 function as a therapeutic opportunity, in contrast to the currently suggested reduction of mutant gene function.

Koeln - Universitaetsklinikum Koeln AöR

Sebahattin Cirak Ph.D

DG Gene discovery of exome-negative muscular dystrophy patients by nextgen RNAseq.

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary The disease causing mutations are known in 50% of the patients with muscular dystrophies. The discovery of disease genes was in the past a time consuming process. It required mapping of the shared genomic region between affected individuals of the specific disease and then the sequencing (decoding) of the genetic code in these regions. Currently the so-called “Exome” sequencing has become available and enabled us in a single experiment to sequence about 80% of the coding region of the human genome. This is leading to the discovery of many disease genes. But still in a large number of patients the mutated genes are escaping discovery. One reason for this is that disease causing mutations can also occur in the so-called noncoding regions of the human genome. These are genetic variants that are involved in the regulation and processing of the genetic information. These so-called noncoding mutations are usually not accessible with exome sequencing. Very recently, a new technique called RNA sequencing has been developed. RNA sequencing is decoding of the entire RNA, the "working copies," or transcriptome, of the human genetic information in the cell. This technique allows us to identify the sequence of the RNA code and but also to determine its quantity. We will extract this RNA from the affected muscle or nerve biopsy of these patients and perform RNA sequencing. This will allow us to investigate the blueprint and identify the mutation.

GREECE

Athens - HELLENIC PASTEUR INSTITUTE

Socrates J Tzartos Ph.D.

RG Diagnosis and characterization of LRP4-MG, a novel myasthenia gravis subtype

$115,011.00	2/1/2014	1/31/2015	Year 2
$115,011.00	2/1/2015	1/31/2016	Year 3

Summary The low-density lipoprotein receptor-related protein 4 (LRP4) presents a novel autoantigen in myasthenia gravis (MG) patients. Autoantibodies against this protein have been recently identified in sera of patients, earlier characterized as seronegative (SN). SN-MG (i.e. MG without identified autoantibodies) presents a serious gap in MG diagnosis and understanding, whereas the identification of LRP4 as antigen will reduce the number of SN-MG patients and will facilitate differential diagnosis of many non-MG patients who need to exclude the presence of MG. Yet, the published frequency of LRP4-MG varies from ~2-50% of SN-MG necessitating further investigation. We will develop and compare highly specific assays (immunoprecipitation and cell based assay) for the best routine diagnosis of LRP4-MG. We will screen characterized MG biobanks from several countries to unequivocally determine the prevalence of LRP4-MG subtype. Most importantly, we will study the LRP4-MG phenotype and the most appropriate treatment, based on the therapeutic history of the identified LRP4-MG patients. Finally, we will study the pathogenicity of LRP4 antibodies in vitro and in vivo, setting the basis for the generation of an animal model for this MG type and for novel therapeutic approaches. In conclusion, we will develop a novel routine diagnostic assay and we will characterize a newly identified MG subtype (LRP4-MG).

ISRAEL

Jerusalem - Hebrew University of Jerusalem

Yosef Gruenbaum Ph.D.

RG The molecular basis of AD-EDMD

$100,003.00	8/1/2014	7/31/2015	Year 3

Summary Autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) is caused by mutations in lamins A/C;

however, the mechanisms by which lamin mutations lead to this disease are currently unclear. Our laboratory has established Caenorhabditis elegans as a powerful system in which to study novel pathways regulated by lamin and its role in human disease. Lamins are evolutionarily conserved and many of the residues that are mutated in AD-EDMD are conserved in Caenorhabditis elegans lamin. Our results also define C. elegans as the only system in which changes in lamin filament assembly in vitro and in vivo can be correlated with the disease phenotypes in vivo. The goal of our current proposal is to expand our studies of the Y59C (Y45C in human) and T164P (T150P in human) lamin EDMD-linked mutations in order to elucidate the molecular mechanisms by which these mutations cause motility and muscle defects. The results of this study should elucidate the underlying mechanisms of the currently enigmatic human AD-EDMD disease, which could identify novel drug targets for developing therapy to treat AD-EDMD.

ITALY

Novara - Department of Translational Medicine, University of Piemonte Orientale "Amedeo Avogadro"- Alessandria, Novara, Vercelli

Nicoletta Filigheddu Ph.D.

RG Exploring the therapeutic potential of unacylated ghrelin for muscular dystrophy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Muscular dystrophies (MDs) are diseases, characterized by the chronic degeneration of muscles, for which no resolutive cure exists. MD patients are currently treated with drugs that relieve the symptoms, but with only moderate and temporary beneficial effects. Acylated and unacylated ghrelin (AG and UnAG, respectively) are circulating hormones induced by fasting. AG stimulates growth hormone release, food intake, and fat accumulation through binding to its receptor GHSR-1a. UnAG does not bind to GHSR-1a and has been considered for many years an inactive product of AG degradation. However, UnAG and AG share many biological effects on several tissues, including a protective activity on both heart and skeletal muscle. AG and UnAG might have potential therapeutic applications for MDs ameliorating the regeneration of skeletal muscle and improving the outcome of cell and gene therapies. We will focus on UnAG because it does not have some potential undesirable effects of AG. We will study the effect of UnAG on muscle regeneration (both in wild type and mdx dystrophic mice) and on transplantation of skeletal muscle satellite cells to repair the damaged muscles.

UNITED KINGDOM

Edinburgh - University of Edinburgh

Lyndsay Murray Ph.D

DG Expression profiling of differentially vulnerable motor neurons in SMA

$50,760.00	5/1/2014	4/30/2015	Year 1
$50,760.00	5/1/2015	4/30/2016	Year 2
$50,760.00	5/1/2016	4/30/2017	Year 3

Summary In SMA motor neurons, which connect the spinal cord to the muscle, die. The part of the neuron which contacts the muscle (neuromuscular junction) appears to be particularly vulnerable with a loss of these connections occurring early in the disease. Furthermore, not all junctions appear equally affected. In a mouse model of SMA, neuromuscular junctions in neck muscles remain healthy, whilst those in abdominal muscles degenerate early. In this study, we will investigate the reason why some motor neurons are more vulnerable than others. Firstly, we will compare gene activity between motor neurons pools in healthy mice to investigate what makes some motor neurons more vulnerable than others. Secondly, we will compare gene activity between SMA and healthy mice in both vulnerable and less vulnerable motor neurons prior to the onset of cell death. This will allow us to investigate the first changes to occur which lead to the death of motor neurons. In order to do this, we will use fluorescent tracers to identify the neurons in the spinal cord and brainstem which connect to muscles which have either high or low amounts of neuromuscular junction loss. We will isolate the motor neurons and use screening methods and powerful software to compare the gene activity. This work will reveal some of the first changes in gene activity which occur before the cell dies and tell us why some cells die while others do not. This work will give us new ideas of how to protect cells and stop them dying.

Oxford - University of Oxford

Kay Elizabeth Davies MA, Ph.D.,

RG Utrophin upregulation for treatment of DMD

	$103,783.00	2/1/2014	7/31/2015	Year 2

Summary It is now well established that increasing utrophin levels in Duchenne Muscular Dystrophy (DMD) patients has the potential to show therapetic benefit. Utrophin in very similar to the missing protein dystrophin and in the mouse model, utrophin can prevent the pathology. We have collaborated with Summit plc in developing a drug which is first in class to increase utrophin levels. This is being taken by Summit plc into Phase I clinical trials (funded partly by MDA). We aim to develop best in class drugs to follow on from this. We have developed a new more sensitive screening assay and have already found new hits that work better than the original drug in tissue culture. These now need to be tested in the mdx mouse model and optimised.

Portsmouth - University of Portsmouth

Darek Gorecki Ph.D.

RG P2X7 receptor as a target for treatment in Duchenne muscular dystrophy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary A molecule called ATP provides the energy muscles need to contract, hence it is found in large quantities inside the tissue and damaged or diseased muscles release large amounts of it. ATP outside the cell becomes a “danger signal” triggering inflammation (body's protective attempt to remove dying tissues to make room for the healing process). ATP sends these signals by interacting with specific proteins on cell surface (called receptors). We have shown that one such ATP receptor (designated P2X7) contributes directly to muscle damage in Duchenne muscles. Notably, inflammation is also an important feature of dystrophic pathology. Thus ATP contributes directly to dystrophic muscle damage and indirectly, through enhancing inflammation. To study whether removing P2X7 receptors could be therapeutic we developed mouse models, which lack the ability to make P2X7 receptors and we found improvements in key disease parameters. Experience with pharmaceutics has shown that receptors are particularly suited for developing “conventional” drug treatments and novel drugs blocking P2X7 are in clinical trials for other diseases. We have sought advice of the Treat-NMD Advisory Committee on Therapeutics who recommended we perform additional pre-clinical studies: Completion of work proposed here explaining the mechanism of this receptor abnormality and showing specific drugs to be effective in the animal model of disease should lead to re-purposing the existing P2X7 medicines to target DMD.

UNITED STATES

ALABAMA

Birmingham - Southern Research Institute

Mark Suto Ph.D.

RG Development of small molecules active at disease onset in ALS

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Amyotrophic lateral sclerosis (ALS) affects 1-2 humans every 100,000. The disease causes degeneration of motoneurons, paralysis and death. The disease is characterized by SOD1 abnormalities that results in an excess of reactive oxygen species (ROS) and mitochondrial sufferance. Transgenic animals carrying SOD1 mutations have been widely used to test experimental agents. We hypothesized that enhancement of MnSOD, spared by SOD1 failure, could ameliorate the disease outcome. After a high throughput screening campaign, we have focused on two molecules which directly activate NF-kB p65 in brain cells via a non-cytokine receptor-mediated mechanism, and up regulated MnSOD expression and activity in brain cells. These molecules have also shown neurotrophic and neuroprotective effects in vitro. Our experiments conducted in animals have shown that administration SR22818 and SR22819 are tolerated and safe in mice. Our data also indicate that the treatment with SR22818 and SR22819 at 20mg/kg daily was associated with significant drug levels in the brain. Moreover, treating SOD1-G93A animals with a similar dose of the compounds at symptoms onset (day 96), caused a significant prolongation of life expectancy, decreased weight loss and improved neurologic symptoms. We propose here the plan to develop novel molecules based on SRI22818 and 22819 with better pharmacodynamic properties to pursue as drugs for the treatment of ALS.

Birmingham - The University of Alabama at Birmingham

Marek Napierala Ph.D.

RG Correction of the Friedreich’s ataxia gene defect using zinc finger nucleases.

$106,130.00	2/1/2014	1/31/2015	Year 2
$103,133.00	2/1/2015	1/31/2016	Year 3

Summary Friedreich's ataxia (FRDA), a severe progressive neurodegenerative disorder, is caused by increasing number of specific DNA sequences termed GAA repeats. This error in DNA leads to the block in the flow of the information from DNA to the RNA leading to deficiency of the final product of the Friedreich's ataxia gene – protein called frataxin. Importantly, this genetic defect causing Friedreich's ataxia does not change the properties of the frataxin, but specifically decreases the yield of frataxin production in patients' cells. Neurons and heart cells are the most sensitive cells to frataxin deficiency, thus during the course of the disease they undergo progressive and irreversible degeneration. In the proposed project we will take advantage of recent technological breakthroughs and generate a collection of neuronal and cardiac cell lines derived from FRDA patients' and controls' skin cells. Subsequently, we will use very specific enzymes called zinc finger nucleases, working as molecular scissors that are uniquely designed to remove disease causing mutation from the Friedreich's ataxia gene. There are two major goals of this research: (i) to create novel, state of the art models of FRDA which will enable identification of molecular targets for therapeutic interventions, (ii) to conduct proof-of-concept studies aimed to repair the mutation leading to FRDA in the patients' cells. This work will generate resources and technology for regenerative therapy of Friedreich's ataxia.

ARIZONA

Phoenix - Barrow Neurological Institute - St. Joseph Med. Ctr.

Jeremy Shefner MD, PhD

HCTG Multi-center, Randomized Controlled Study of Diaphragm Pacing for ALS

$125,000.00	1/1/2015	3/1/2015	Year 4

Summary Amyotrophic lateral sclerosis (ALS) often results in breathing difficulties. This project will test whether electrical stimulation of the diaphragm (the main breathing muscle in the chest) is of benefit to people with ALS. It is unknown whether treatment of breathing muscle weakness with electrical stimulation of the diaphragm muscle with the NeuRx® Diaphragm Pacing System (DPS) slows the progression of the disease. This study is being done to figure out if DPS treatment will improve breathing function or prolong life span in people with ALS.

Tucson - Arizona Board of Regents, University of Arizona

Henk Granzier PhD

RG Improving Muscle Function in Nebulin-based Nemaline Myopathy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Nemaline myopathy (NM) is the most common non-dystrophic congenital myopathy, with mutations in the nebulin gene (NEB) accounting for ~50% of NM cases. Nebulin is a giant sarcomeric protein that is coextensive with the thin filament. Insights in nebulin's functions made a leap forward when nebulin KO mouse models were made and with the recent publication of a mouse in which Neb exon 55 is deleted to model a founder mutation frequently seen in NM patients. Although these models have greatly helped in providing insights in nebulin's functions, their phenotype is much more severe than that of NM patients (mice die within days after birth) limiting their usefulness. The severe phenotype of mice might be due to the fact that nebulin is virtually absent unlike in patients where ~10-20% of the normal nebulin levels often remain. To overcome these shortcomings we made a conditional nebulin KO model (NEB cKO). Pilot studies reveal that when nebulin deletion is achieved by expressing Cre recombinase driven by the MCK promotor (MKC-Cre cNEB KO), mice survive much longer than full NEB KO mice (the oldest mice have reached ~ 2 mo of age), that small level of expression of nebulin persists (~15% of maximal) and that muscle weakness is severe. These features resemble closely those of NM patients. Here we proposed to use MCK-Cre cNEB KO mice and study the mechanistic basis of muscle weakness (Aim 1) and test the effect of therapeutics (Aims 2 and 3).

Archi Joardar

B2I Genetic and Translational Approaches in a Drosophila Model of ALS Based on TDP-43

$60,000.00	8/1/2014	7/31/2015	Year 3

Summary Amyotrophic Lateral Sclerosis (ALS) is a progressive neurological disorder that leads to paralysis and death. The pathological features of this devastating disorder include motor neuron loss and muscle atrophy. With the recent identification of cellular inclusions containing TDP-43 protein plus the discovery of mutations in TDP-43 in patients, this protein has emerged as a common denominator in a significant fraction of ALS

cases. We have found that human TDP-43 carrying mutations identical to those found in ALS patients, when expressed in fruit fly motor neurons, leads to neuroanatomical and locomotor defects that mimic clinical manifestations of the human disease. Testing this Drosophila model of ALS against a large panel of FDA-approved drugs, we identified some that alleviated neurotoxicity of human TDP-43 in the fly. Here we propose to further develop a subset of those compounds, which target a nuclear protein involved in cellular metabolism. We will use genetic interactions to determine what aspects of TDP-43 neurotoxicity are mediated by our newly discovered candidate target. These whole organism data will be translated to mammalian systems using in vitro assays in a collaboration with Sanofi. Through this collaborative approach, involving academic research in an animal model of ALS combined with proven drug discovery tools in an industry setting, we are well positioned to discover novel therapeutic targets and strategies for ALS that might eventually lead

Daniela Zarnescu Ph.D.

RG	Deciphering the role of insulin signaling in ALS			
	$135,000.00	2/1/2014	1/31/2015	Year 2
	$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Amyotrophic Lateral Sclerosis (ALS) is a fatal neurological disorder characterized by motor neuron loss and muscle atrophy. With the recent identification of cellular aggregates containing TDP-43 plus the discovery of TDP-43 mutations in patients, this protein has emerged as a common denominator for the majority of ALS cases. We have found that human TDP-43 carrying mutations identical to those found in ALS patients, when expressed in fruit fly motor neurons, leads to neuroanatomical and locomotor defects that mimic clinical manifestations of the human disease. Testing this model of ALS against a large panel of FDA-approved drugs, we identified several that rescued the lethality of human TDP-43 in the fly. These drugs include several categories currently prescribed for diabetes, which are known to improve cellular function by influencing the insulin signaling pathway. To further test the therapeutic value of these antidiabetic drugs and to investigate the role of the insulin pathway in ALS we will take a combined pharmacological and genetic approach using our fruit fly model. Given that our candidate drugs are already approved for use in humans, our work will help determine whether they could be prescribed for the treatment of ALS patients and could aid in the development of future therapies.

CALIFORNIA

Davis - The Regents of the University of California (University of California Davis)

David Paul Richman M.D.

RG	Pathogenesis of Anti-MuSK Myasthenia			
	$137,500.00	2/1/2014	1/31/2015	Year 2
	$137,500.00	2/1/2015	1/31/2016	Year 3

Summary MuSK myasthenia (AMM), a new form of myasthenia (MG), appears to be caused by auto-antibodies (Abs) to a different protein in the nerve/muscle synapse than that targeted in standard MG. In AMM the weakness occurs in a more restricted group of muscles, which also undergo wasting. Also, AMM is more difficult to treat because many usual MG treatments are not effective. We have developed an animal model of AMM by immunizing Lewis rats with purified MuSK. These animals produce large amounts of Abs to MuSK and develop a severe form of the disease, experimental anti-MuSK myasthenia (EAMM), which is fatal within 27 days of immunization. The characteristics of AMM are faithfully reproduced, most importantly the marked muscle wasting. Therefore, EAMM provides a means to determine how Abs induce the disease, thereby identifying targets for treating AMM, especially the muscle wasting. To accomplish this, we will analyze systems within muscle that lead to either increased growth or muscle wasting to determine the mechanisms involved in AMM, information that may also be applicable to other muscle diseases involving wasting.

La Jolla - The Regents of the University of California, San Diego

Adam Jeffrey Engler Ph.D.

RG	Mechanically programmed adipose-derived stem cells to treat muscular dystrophy			
	$130,000.00	8/1/2014	7/31/2015	Year 3

Summary The major challenge of restoring muscle contraction to patients with muscular dystrophy has been to deliver cells that can overcome the fibrotic, stiff cell niche of the degenerated muscle, avoid converting into intramuscular fat, and fuse with muscle fibers. While several cell sources have been proposed, most adult stem cell sources are not abundant for clinically viable treatment, cannot fuse into dystrophic muscle, or cannot restore function. However, we have mechanically induced adipose-derived stem cells (ASCs) to become muscle, and they can maintain their fused muscle state in dystrophic muscle-like environments in vitro. In this project, we will first understand the differences between how ASCs and other cell sources, including satellite cells and intramuscular fat, sense and respond to stiffness, which enables ASC-derived

muscle and intramuscular fat to remain their fates despite the presence of a stiff environment instructing the cells to become other tissues. We will then assess their fusion potential with host animals and determine their ability to form ex vivo innervated tissue constructs in bioreactor cultures that mimic dystrophic muscle. Finally, we will perform intramuscular injections of ASC-derived myotubes and assess engraftment, dystrophin expression, and restoration of degenerated muscle function. Successful validation of functional muscle restoration using ASC-derived muscle will lead to larger animal studies and potential clinical translation.

Albert La Spada M.D., Ph.D.

RG SBMA motor neuron degeneration: molecular basis and therapy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Spinal and bulbar muscular atrophy (SBMA, Kennedy's disease) is an adult onset neuromuscular disorder affecting only men. We have made considerable progress in understanding why motor neurons are dying in this disease, and now wish to continue our studies to confirm the mechanistic basis of the motor neuron degeneration, as well as perform preclinical trials in mice to test exciting new therapies to treat SBMA. Toward this end, we have created a highly representative mouse model of SBMA and have produced neuron cell culture models of SBMA. In the next stage of our SBMA research work, we will use these models to examine the role of altered metabolism in SBMA disease pathogenesis, and we will determine if altered metabolic processes could be used to track the progression of SBMA motor neuron disease through a metabolic biomarker. We will also test if impaired protein turnover in SBMA stems from altered function of a particular master regulatory factor, and we will develop drug therapies to promote the function of this regulatory factor. Finally, using an emerging technique known as antisense oligonucleotide “knock-down”, we will determine if reduction of mutant androgen receptor gene expression is a viable therapy for SBMA, comparing peripheral delivery with central nervous system delivery, as work funded by the MDA in our lab has shown that termination of disease gene expression in muscle can prevent SBMA in mice.

La Jolla - The Scripps Research Institute

Matthew Disney Ph.D.

RG Identification & Optimization of Small Molecules Targeting r(CCUG)exp in DM2

$120,908.00	2/1/2014	1/31/2015	Year 2
$120,908.00	2/1/2015	1/31/2016	Year 3

Summary Myotonic dystrophy type 2 (DM2) is a form of muscular dystrophy caused by a defective RNA. We have previously used our expertise in understanding how drug-like compounds interact with RNA to develop compounds that improve defects associated with a similar disease, myotonic dystrophy type 1, in both cellular and animal models. We will apply our knowledge to identify drug-like compounds that improve DM2 defects: 1.) We previously designed compounds that are effective in vitro. Therefore, we will optimize and test these compounds for improving DM2-associated defects in cell culture models. 2.) We will leverage our expertise in understanding how drugs bind to RNA to identify new lead compounds for treating DM2. Compounds will be tested in vitro and then in cell culture models of DM2.

David Samuel Gokhin Ph.D.

DG Structure, Regulation, and Function of Gamma-Actin in the Sarcoplasmic Reticulum

$60,000.00	8/1/2014	7/31/2015	Year 3

Summary The skeletal muscle sarcoplasmic reticulum (SR) is a cellular membrane system that houses the calcium reservoir for muscle contraction and is critical for normal muscle function. Membrane fragility in Duchenne muscular dystrophy is associated with aberrant calcium leakage from the SR. The SR also contains cytoplasmic gamma-actin filaments, which act as molecular scaffolds that mechanically undergird the SR and link the SR to the myofibrils, which are the force-generating units of muscle contraction. Gamma-actin filaments are biological polymers whose ends are protected by tropomodulin 3 (Tmod3) capping molecules, splinted along their sides by rod-like tropomyosin (TM) molecules, and tethered to the SR via a specialized linker protein (small ankyrin 1.5). This project will explore the hypothesis that gamma-actin, stabilized by Tmod3, regulates the structure and function of the skeletal muscle SR. First, I will use purified proteins to study how gamma-actin filaments are linked to the SR and how these links are stabilized by Tmod3, TM, and other scaffold elements of the SR. Next, I will investigate SR structure, calcium transport, and intracellular SR-myofibril linkages in muscles from normal mice whose muscles are missing Tmod3, missing gamma-actin, or contain excess amounts of gamma-actin. Finally, I will examine the significance of elevated SR-associated gamma-actin in the disease course of a validated animal model of Duchenne muscular dystrophy, the mdx mouse.

Sunitha Rangaraju Ph.D

DG Improving ALS phenotypes by targeting aging pathways

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary Our aim is to identify molecules that could be developed into therapeutics for amyotrophic lateral sclerosis (ALS). ALS is an age-related neuromuscular disease whose progression occurs with aging and is thought to be driven by the aberrant aggregation of certain proteins, which then leads to the collapse of protein homeostasis. We reasoned that, molecules that delay aging may prove as effective therapeutics for ALS. We previously screened over 89,000 molecules for those that delay aging and extend lifespan of C. elegans, a small worm that is widely used to study aging. We identified over 100 molecules that extend lifespan, which I am currently testing on a C. elegans model of ALS, to identify potential therapeutic leads. Similar to humans, the worm model of ALS shows protein aggregation, movement defects, reduction of neuron-to-muscle signals, and a shorter lifespan. These ALS-like phenotypes result from the expression of a mutant form of a human disease-causing gene called SOD1. So far, I have successfully identified 4 molecules that extend lifespan of the ALS worms. We hypothesize that these molecules will either reduce SOD1 aggregation, or mitigate its negative effects on the animal's physiology. In this project, I will continue to screen for molecules that extend lifespan of the worm ALS model, I will test the current and future hits for their ability to suppress the above mentioned ALS phenotypes, and finally test the most promising molecules in mouse models of ALS.

La Jolla - University of California, San Diego - Health Sciences

Constanza Cortes Ph.D

DG TFEB-mediated autophagy dysregulation in SBMA

$58,491.00	8/1/2014	7/31/2015	Year 2
$59,648.00	8/1/2015	7/31/2016	Year 3

Summary Autophagy is a pathway that cells use to get rid of misfolded proteins and damaged organelles. In this project, we will study the role of autophagy dysfunction in spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS). We will expand our understanding of neuronal autophagy, a field that remains obscure and poorly developed, by applying classical immunoflourescence, electron microscopy and pull-down assays in our models of SBMA. In parallel, we will also use powerful genetic mouse models of SBMA to develop autophagy-intervention therapeutics and test these approaches in vivo to determine the feasibility of manipulating autophagy as a therapeutic strategy for motor neuron disease.

Los Angeles - The Regents of the University of California, Los Angeles

Linda Gwen Baum M.D., Ph.D.

RG The human skeletal muscle cell glycome - structures and functions

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Extensive study of mouse models of Duchenne muscular dystrophy has yielded critical information about how loss of dystrophin affects animal muscle cell biology, including glycosylation of cellular glycoproteins essential for proper cell function. However, it is now very clear that human cellular glycosylation machinery is different from rodent and rabbit cell glycosylation machinery; while recent studies have found that altering cell glycosylation in mouse muscle cells can improve muscle cell function, such approaches may have limited value in human cells that use different glycosylation machinery. In this project, we will 1)profile glycan structures on human muscle cells derived from patients with DMD and their parents; 2)determine the biochemical events that create these specific glycans on human muscle cells; 3)identify specific glycans that can be manipulated to enhance muscle cell function and perform high-throughput screening for compounds that enhance expression of these function-related glycans on human muscle cells, to identify lead compounds for new human therapeutics. This will require a comprehensive approach that has not been used previously, but which we have successfully developed already with mouse muscle cells and are optimally poised to apply to human muscle cells, using the resources of the Center for Duchenne Muscular Dystrophy at UCLA.

Carmen Bertoni Ph.D.

RG Gene Editing of Dystrophin for the Treatment of Duchenne Muscular Dystrophy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Duchenne Muscular Dystrophy (DMD) is a genetic disorder caused by the absence of a protein called dystrophin. To date there is no effective cure for DMD and the best option to treat the disease is to restore

expression of dystrophin. Our research group has pioneered the use of gene editing strategies for the dystrophin gene to permanently correct the DNA: the source of the problem. DNA contains the information needed by every cell, including muscles, to function properly. In DMD patients the DNA that makes up the dystrophin gene contains errors. We can use oligonucleotides to let the muscle know of those errors and give the opportunity to the cell that compose each muscle to correct the mistake. We have shown that oligonucleotides can treat mouse models for DMD. In this proposal we intend to compare the efficacy of oligonucleotides to that obtained using a new generation of gene editing tools called Transcription Activator–Like Effector Nucleases (TALENs) and Transcription Activator–Like Effector Nickases (TALENickases) and determine whether we can increase the efficiency of the repair to levels suitable to treat the disorder. Comparison will be performed at first in culture using muscle cells isolated from a mouse model for DMD and then in a DMD mouse model to determine the feasibility of using this technology in patients. Each one of these steps is necessary to ensure a safe and effective treatment to human patients.

Rachelle H. Crosbie-Watson Ph.D.

RG Evaluation of sarcospan treatment in muscular dystrophy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Loss of functional dystrophin protein in DMD results in reduced muscle membrane stability, with muscle fiber necrosis and fibrosis. It is well established that utrophin can replace dystrophin and stabilize the muscle cell membrane to ameliorate muscular dystrophy. Over the last ten years, intense efforts have been narrowly focused at identifying molecules that could increase utrophin mRNA transcripts; however, these efforts have not yielded any viable therapies. We have discovered a novel method that improves sarcolemma stability and adhesion. The current proposal is aimed at testing the mechanisms and feasibility of this novel approach in animal models of DMD, AR-LGMD, and CMD. The outcome of these experiments will contribute to a better mechanistic understanding of the molecular events contributing to the ability of sarcospan to alter expression of proteins at the cell surface and alter the course of dystrophic pathology and reveal the efficacy of sarcospan for the treatment of other muscular dystrophies.

Bennett Novitch Ph.D.

RG Developmental mechanisms controlling respiratory motor functions

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Our ability to breathe, move, and interact with the world depends on the function of motor neurons in the spinal cord that make connections to various muscle groups in the body to regulate muscle activity. Numerous neurodegenerative diseases such as spinal muscular atrophy (SMA), amyotrophic lateral sclerosis, and spinal bulbar muscular atrophy result from a breakdown in the communication between motor neurons and muscle cells, leading to the death of the neurons, paralysis, and a foreshortened patient lifespan. Most fatalities associated with motor neuron disease result from respiratory failure, and many patients require mechanical ventilation for their survival. Currently there are no effective treatments for these diseases, as very little is known about the underlying mechanism that results in motor neuron death. This proposal will test the hypothesis that the loss of respiratory motor neurons in early onset motor neuron diseases such as Type 1 (severe) SMA, can be attributed to defects in the process by which respiratory motor circuits are assembled during fetal development. Our study will provide new insights into the root causes of SMA and potentially lead to the discovery of new therapeutic targets. Moreover, by studying the process by which respiratory motor circuits are initially formed, we will gain vital information on how this activity may be recapitulated to rebuild damaged circuits to help patients maintain their ability to breathe independently.

Melissa Spencer Ph.D.

RG Mechanisms involved in calpainapathies

$130,000.00	8/1/2014	7/31/2015	Year 3

Summary Limb girdle muscular dystrophy type 2A due to mutations in the gene encoding calpain 3 n(C3) is one of the most prevalent LGMDs. Our previous studies have created genetically modified mice to understand the biological function of calpain 3 and have demonstrated that muscles lacking calpain 3 do not grow properly. Concomitantly, we have identified a signaling pathway that is defective in muscles lacking calpain 3. In this investigation, we will determine whether loss of this signaling pathway is the basis for the impaired growth in LGMD2A, and we will determine if this pathway can be pharmacologically targeted for therapy.

Julio Vergara Ph.D.

RG Calcium release alterations in malignant hyperthermia and central core disease

$100,000.00	8/1/2014	7/31/2015	Year 2

$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Malignant hyperthermia (MH) susceptibility and central core disease (CCD) result mostly from mutations in the gene encoding the ryanodine receptor Ca2+ release channel (RyR1). These disorders share gross abnormalities in Ca2+ homeostasis, but differ in that individuals with CCD display muscle weakness, while those with MH develop rigidity and hyperthermia when exposed to triggering agents (e.g. halothane). Knockin mice permit to study the mechanisms underlying the intertwined MH/CCD human pathology. This is the case of the R163C and T4826I mice, which express diverse (and prevalent) MH/CCD human mutations of the RyR1; however, there is limited information about the detailed alterations of Ca2+ signaling in muscle fibers from these transgenic mice. Major pending questions are: Why do mutated RyR1 channels behave asymptomatically under basal conditions, but predispose fibers to triggering agents that lead to fulminant MH episodes? And, how does dantrolene prevent fulminant MH episodes? We will investigate these issues, using advanced electrophysiological and optical methods, in fibers isolated from R163C and T4821adult mice under “triggered” and “non-triggered” conditions (with and without halothane). Our studies comparing and contrasting the alterations in Ca2+ signaling observed in muscle fibers of R163C (heterozygous) mice with those of T4826I (heterozygous and homozygous) mice will also provide novel insights on the effects of dosage and penetrance in MH/CCD RyR1 mutations.

Palo Alto - Stanford University

Aaron Gitler Ph.D.

RG Defining a novel role of profilin 1 in ALS pathogenesis

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Mutations in the profilin 1 gene (PFN1) were recently identified as a cause of ALS. The role of PFN1 in ALS and the mechanism by which mutations cause disease is unknown. Yeast cells also have a PFN1 gene and we found that we could replace this yeast gene with the human one. This has allowed us to compare and contrast WT and mutant PFN1 and to help determine how the PFN1 mutations might cause ALS. We will systematically test all of the reported PFN1 mutations in this yeast assay. This will help to classify candidate variants into functional categories and aid in prioritizing specific variants for the development of animal models. These results could also aid in clinical interpretation of PFN1 genetic testing. We will also use the yeast model system to perform unbiased genetic screens for genes that interact with PFN1. We reason that the types of genes and pathways that we identify will provide insight into potential novel cellular functions for PFN1. Not only will this tell us what PFN1 normally does but it might help to suggest targets for therapeutic intervention. We made an unexpected and exciting finding, connecting PFN1 to stress granules, tiny cellular factories that store and process RNA molecules. Stress granules have been associated with ALS and now our finding expands this role and suggests an important new function for PFN1 as a stress granule regulator. We will define this function and determine how ALS-linked PFN1 mutations impair this function.

Pasadena - California Institute of Technology

David Chan MD/PhD

RG Mitochondrial dynamics as a protective factor in mitochondrial myopathies

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Skeletal muscle has enormous energy demands. This tissue is therefore highly dependent on the function of its mitochondria, organelles that provide the bulk of cellular energy. Mutations in the mitochondrial genome impair mitochondrial function and lead to a large group of severe diseases termed mitochondrial myopathies. In these diseases, there is poor skeletal muscle function due to reduced energy production. Our previous work has shown that mitochondria are dynamic organelles that continually fuse and divide. These processes protect mitochondrial function and have been shown to be particularly important when mitochondrial DNA mutations occur. We have generated several lines of mutant mice that have defects in mitochondrial fusion or division. We will use these mice to study the role of mitochondrial fusion and fission in skeletal muscle, with an emphasis on understanding whether specific muscle fiber types depend more critically on these processes. In addition, we will the mechanism that controls the different properties of specific types of muscle fiber cells. These studies may improve our understanding of the pathogenesis of mitochondrial myopathies.

COLORADO

Aurora - University of Colorado Denver, AMC and DC

Kurt Beam Ph.D.

RG Analyzing DHPR-RyR1 interactions in a reduced system

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Skeletal muscle contraction, which is essential for the ability to move and breathe, is triggered by an electrical signal. This process, termed excitation-contraction coupling, depends on two key proteins: the dihydropyridine receptor (DHPR) which is located in the membrane surrounding the muscle cell, and the ryanodine receptor (RyR1) located inside the cell. Mutations of these proteins result in serious muscle diseases in humans, including hypokalemic periodic paralysis and central core disease. In this project, we will define how the DHPR and RyR1 interact with one another and why mutations cause these human muscle diseases.

Boulder - The Regents of the University of Colorado d/b/a University of Colorado at Boulder

Leslie Leinwand Ph.D.

RG Mechanisms of Myopathy Caused by Mutations in the Myosin Rod

$112,925.00	2/1/2014	1/31/2015	Year 2
$112,925.00	2/1/2015	1/31/2016	Year 3

Summary We will study an inherited skeletal muscle disease and test a novel therapeutic approach. The disease is called Laing distal myopathy and it is caused by mutations in the muscle motor protein called myosin. The name of the gene is the beta-myosin heavy chain, the major muscle motor protein expressed in human heart and slow skeletal muscle fibers. After measuring the impact of the mutated proteins in different cell and animal models, we will test inactivation of the mutant myosin as a treatment.

Fort Collins - Colorado State University

Eric Ross Ph.D.

RG Aggregation and toxicity of ALS- and IBM-associated prion-like domains

$121,000.00	2/1/2014	1/31/2015	Year 2
$121,000.00	2/1/2015	1/31/2016	Year 3

Summary Protein aggregation is associated with numerous human diseases including amyotrophic lateral sclerosis (ALS) and some forms of inclusion body myopathy (IBM). Prions are infectious protein aggregates. Numerous human proteins contain prion-like domains (PrLDs) – domains with compositional similarity to yeast prion domains. Remarkably, in the past few years, six of these proteins have been linked to some forms of ALS or IBM. However, despite the importance of these PrLDs in human disease, the basis for their aggregation and toxicity is still poorly understood. In this proposal, we will use a combination of yeast and Drosophila genetics and in vitro assays to rigorously define how the amino acid sequence of PrLDs contributes to aggregation and toxicity. These studies will provide insight into the causes of these diseases, facilitate the identification of potential drug targets for therapeutic intervention, and improve our ability to identify other disease-associated PrLDs.

DISTRICT OF COLUMBIA

Washington - Children's Research Institute (CNMC)

Eric Hoffman Ph.D.

RG Asynchronous remodeling: A force driving failed regeneration in DMD.

$105,339.00	2/1/2014	1/31/2015	Year 2
$107,577.00	2/12/2015	1/31/2016	Year 3

Summary The goal of the proposed research is to determine why Duchenne muscular dystrophy (DMD) is a progressive disease, and then use this knowledge to design better treatments. In normal individuals, muscle can be injured and repaired. The process is started by the single injury, and the muscle undergoes a coordinated process of repair that takes 2 wks. Our model is that muscle repair in DMD is asynchronous. Namely, different regions of DMD muscle start the repair process at different times, and neighboring regions of the muscle get disoriented as to which time point in the 2 wk time frame of repair they are in. This results in inappropriate signals, and failed regeneration. The corollary to this model is that drugs able to re-synchronize muscle repair in DMD should be effective. We present experimental data consistent with this model, and propose that glucocorticoids and the newer VBP15 drug are in effect 're-synchronization' agents in DMD.

Terence Anthony Partridge Ph.D.

RG Role of satellite cells and pericytes in maintenance of dystrophic muscle

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary To combat diseases that involve loss of muscle, an important strategy is to facilitate the cellular mechanisms that maintain and repair muscle. This is especially important in the case of diseases like Duchenne muscular dystrophy, where muscle tissue destruction goes on throughout life. We now have methods that permit us to mark the main categories of cell that have been identified as sources of the repair mechanism and will use these to determine how large a role each plays in long-term repair of muscle in the mdx mouse model of muscular dystrophy. We will also use these markers to purify cells that exhibit different behaviors in the process of muscle repair and will identify the mechanisms behind these differences. By analysis of the patterns of gene expression, we will identify the signaling pathways to which they respond. This will inform us as to which cell-types we should be grafting or encouraging in their function so as to optimize the repair process.

Terence Anthony Partridge Ph.D.

RG Pre-clinical efficacy testing of Tricyclo antisense oligonucleotides for DMD

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary For Duchenne muscular dystrophy (DMD), exon-skipping is one of the most promising approaches. It works by excluding the mutated part of the gene from the messenger RNA copy (the blueprint) of the gene that is translated within the muscle fiber into the dystrophin protein. This is done by giving an antisense oligonucleotide, short length of a DNA-like structure, that masks the sites that are normally used to include this part of the gene. Removal of this damaged region leads, in most cases, to the production of a slightly smaller dystrophin protein that retains part of the function of normal dystrophin, which should transform the clinical picture from the severe pathology associated with DMD to a milder disease that resembles Becker muscular dystrophy. The main problem with the present antisense reagents is that they do not readily enter all muscles , least of all the heart muscle. We propose to test a new chemical form that appears to enter heart and skeletal muscle. We will test this on a dystrophic mouse that has a mutation in the region of the dystrophin gene where many human DMD mutations occurs. This will allow us to test a larger variety of antisense agents than is possible with the dystrophic mouse we have used up to now.

Washington - Childrens Research Institue

Jyoti Kumar Jaiswal Ph.D.

RG Analysis of VBP 15 as a drug based therapy for treating dysferlinopathy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Dysferlinopathies are muscle wasting disorders where mutations in dysferlin gene cause a deficit of this protein. Dysferlin is a membrane associated protein that is expressed in sarcolemma and inflammatory cells. Deficit of this protein causes poor repair of injured sarcolemma as well as chronic muscle inflammation. Use of agents that have anti-inflammatory ability such as prednisone, are not an effective therapy for dysferlinopathy. This could in part be due to the detrimental effect of prednisone on the primary deficit of dysferlinopathic myofibers namely, poor ability of dysferlinopathic myofibers to heal. Thus, a better therapeutic for dysferlinopathy would be agents that improve the healing ability of the myofibers. We have identified a compound VBP15 that causes the treated myofibers to exhibit significantly improved repair. VBP15 is also a potent anti-inflammatory agent that avoids the deleterious effects associated with the use of other steroidal anti-inflammatory drugs. In the proposed work we will assess its preclinical efficacy of VBP15 for treating dysferlinopathy.

Washington - The George Washington University

Maria Chiara Manzini Ph.D.

RG Unraveling the phenotypic variability of alpha-dystroglycanopathies

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Alpha-dystroglycanopathies comprise the most severe forms of congenital muscular dystrophy and are often associated with profound cognitive deficits. A dozen genes regulating dystroglycan glycosylation have been

involved in these disorders, but 50% of cases remain unexplained. In addition to this extreme genetic heterogeneity, affected individuals with mutations in the same gene display variable clinical presentation, ranging from perinatal mortality to limb-girdle muscular dystrophy. Such heterogeneity greatly hinders genetic testing and therapy development, and a better understanding of the etiology of these disorders is needed including the identification of the molecular mechanisms responsible for clinical variability. The application of next generation sequencing technologies has been very successful for the identification of novel alpha-dystroglycanopathy genes in combination with in vivo functional validation in the zebrafish. In the proposed research we will apply this gene identification strategy to a cohort of unexplained cases and we will extend the use of the zebrafish embryo as a model to study phenotypic variability and efficiency of different therapeutic approaches. These studies will not only provide a molecular diagnosis for additional alpha-dystroglycanopathy cases, but will also determine how different mutations affect dystroglycan and how therapeutic strategies may vary depending on the affected enzyme.

FLORIDA

Coral Gables - Miller School of Medicine of the University of Miami

Gavriel David Ph.D., M.D.

RG Calcium pathways in peripheral myelinated axons

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary In demyelinating neuropathies, the normal ensheathment of axons by myelin is disrupted, causing debilitating motor and sensory deficits. These symptoms are caused by dysfunction and degeneration of axons. To understand the link between demyelination and axonal dysfunction, we use axons from mice with genetic defects of myelin formation, that also occur in Charcot-Marie-Tooth disease. In initial work, we discovered that phrenic nerve motor axons with disrupted myelin experience abnormally-large increases in intracellular [Ca2+] during action potential activity. Since Ca2+ over-load often contributes to neuronal death, we will identify Ca2+ elevation pathways that become unmasked/augmented when the myelin is disrupted, and determine if axonal proteins become damaged by the calcium-activated enzyme calpain. Since demyelinating neuropathies afflict both motor and sensory axons, we will test if Ca2+ handling in normal and demyelinated axons differs between motor (ventral root) and sensory (dorsal root) axons. Some patients with demyelinating neuropathies have painful muscle cramps caused by spontaneous action potentials in motor axons. We will record intracellular voltage to test if Ca2+ modulated K+ channels contribute to generation of this abnormal electrical activity. By revealing the basic biology of Ca2+ in peripheral axons with normal and damaged myelin, our results will contribute to understanding and possibly treating the axonal damage in demyelinating neuropathies.

Carlos T. Moraes Ph.D.

RG Reducing the levels of mtDNA mutations by mitochondrial nucleases

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Mitochondrial DNA (mtDNA) mutations are major causes of mitochondrial myopathies. The clinical phenotypes range from a relatively mild ocular myopathy (ophtalmoplegia and ptosis) to a muti-organ devastating conditions. Most often, mtDNA mutations are present in a heteroplasmic condition, where the mtDNA with a mutation co-exists with the wild-type one. At the cellular level, a biochemical defect is evident only when the levels of the mutant mtDNA population are very high (more than 80%). This is also observed in patients' tissues. In this project, we propose to reduce the levels of mtDNA with mutations in cultured human cells and mice by using specific DNA cleaving enzymes targeted to mitochondria (mitoTALEN). Such reduction has the potential to be curative and we have preliminary data providing proof that the approach works.

Gainesville - University of Florida

Laura P.W. Ranum Ph.D.,

RG Molecular Effects of Repeat Associated Non-ATG Translation in Myotonic Dystrophy

$138,364.00	2/1/2014	4/30/2015	Year 3

Summary We have discovered a new type of translational mechanism in which microsatellite repeat sequences direct the expression of proteins in all three reading frames in the absence of the normal regulatory signals. We call this process repeat associated non-ATG (RAN) translation. We have evidence that this process results in the expression of unexpected mutant proteins in myotonic dystrophy. Specifically, we have data showing the

expression and accumulation of a homopolymeric polyglutamine expansion protein in DM1 patient cells and mice. The goal of this project is to better understand the potential effects of RAN-translation in myotonic dystrophy.

Maurice Swanson Ph.D.

RG Circadian Clock Dysregulation in Myotonic Dystrophy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary The most common form of adult-onset muscular dystrophy, myotonic dystrophy (DM), is generally classified as a muscle disease although it is a multi-systemic disorder. Since DM patients and their families have noted that one of the most debilitating aspects of this disease is hypersomnia or excessive daytime sleepiness, the goal of this study is to define the molecular mechanisms underlying abnormal sleep regulation in DM. The research plan is based on the hypothesis that muscleblind-like (MBNL) proteins play essential functions in circadian clock and sleep regulation and DM-associated sleep problems result from loss of MBNL function by the expression of toxic RNAs. Mouse and cell models will developed to study how normal circadian rhythms are altered by inhibition of MBNL activity ad identify the key circadian cycle regulatory genes that are affected in DM. This study should provide significant new insights into the cellular basis for abnormal sleep patterns in DM and promote the development of novel therapeutics to treat excessive daytime sleepiness.

Maurice Swanson Ph.D.

SG 10th International Myotonic Dystrophy Conference

$10,000.00	3/1/2015	6/30/2015	Year 1

Summary Myotonic dystrophy (DM) is the most common form of adult-onset muscular dystrophy with congenital to late adult-adult onset. DM is an important muscular dystrophy in part because of the highly variable and multi-systemic phenotype of this disease. The first meeting of the International Myotonic Dystrophy Consortium (IDMC-1) occurred in Paris in 1997, and subsequent meetings have been held throughout the world every two years. These biennial meetings have had a profound impact on the development of scientific insights into DM pathogenic mechanisms and provided a collaborative venue that has resulted in rapid progress in the DM field. This proposal is designed to provide financial support for the 10th IDMC meeting, which will return to Paris for the first time since the inception of the IDMC community. IDMC-10, which will bring together a diverse group of basic and clinical research scientists together with DM patients and their families, will cover DM disease mechanisms and the development of novel cell and animal disease models. However, a greater emphasis will be placed on current antisense oligonucleotide (ASO) therapy trials as well as the development of future small molecule and other therapeutic strategies.

Miami - University of Miami School of Medicine

Stephan Zuchner M.D.

RG Gene identification in axonal CMT families

$130,000.00	8/1/2014	7/31/2015	Year 3

Summary Charcot-Marie-Tooth disease (CMT) comprises a genetically heterogeneous set of inherited peripheral neuropathies. CMT affects 1 in 1,250 – 2,500 individuals cumulatively making it one of the most widespread inherited diseases. No treatments are available. By identifying the causative genes research can increasingly develop more specific hypotheses about CMT and other diseases. This will ultimately allow for development of therapies. Thus far, more than 50 different CMT genes have been reported; yet, these genes explain only ~30% of the axonal forms of the disease, designated CMT type 2. However, we and other expect more than 100 genes to be responsible for CMT. With this many genes the molecular “puzzle” will be solvable. We are proposing to apply the latest genomic technology to identify these missing genes and also, importantly, study the new genes in yeast, zebrafish and or mammalian cell models to understand their specific molecular function.

GEORGIA

Atlanta - Emory University

Ayan Banerjee Ph.D.

DG Regulation of PABPN1: Implications for Oculopharyngeal Muscular Dystrophy

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary The muscle disease called oculopharyngeal muscular dystrophy (OPMD) typically afflicts patients in their 4th or 5th decade of life and causes the most problems with eyelid muscles and muscles required for swallowing. Although we know what gene (the nuclear nuclear poly(A)-binding protein 1, or PABPN1 gene), is altered in

this disease, we do not understand why this change causes a muscle disease and we also do not currently have any treatment for this fatal disease. The goal of this proposal is to understand how the protein that is defective in OPMD, PABPN1, is regulated. If we can understand how the function of PABPN1 can be modulated, we may be able to develop new therapeutic approaches to treat OPMD.

Gary Bassell Ph.D.

RG RNA localization defects in SMA

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Spinal muscular atrophy (SMA) is the most common inherited cause of infant death, characterized by a neurodegenerative process affecting primarily motor neurons of the spinal cord. This autosomal recessive disease is caused by deletions or mutations of the survival motor neuron gene (SMN1) that encodes for the SMN protein. A major gap is our poor understanding of the pathomechanism whereby motor neurons are selectively vulnerable to SMN deficiency and lead to axonal pathology and neurodegeneration in SMA. The objectives of this proposal are to characterize non-canonical functions of SMN in neurons related to the localization of mRNAs in neuronal processes and their regulation by neurotrophin signaling. This research is envisioned to have important implications for future therapeutic strategies in SMA that use genetic and/or pharmacologic methods to manipulate axonal mRNA regulation.

Jonathan D. Glass MD

RRG Clinical, Pathological, and Proteomic correlations in ALS (2013 Night of Hope)

$135,384.00	1/1/2015	12/31/2015	Year 1

Summary Patients with Amyotrophic Lateral Sclerosis (ALS) show very wide variability in age of onset, rapidity of progression, and body regions affected. Indeed, it may be that ALS is really several diseases with similar clinical features. If this is the case, then therapeutic trials designed to treat ALS may fail because the treatment may be addressing more than one disease mechanism. It is essential for the ALS research community to identify subgroups of ALS patients with common disease mechanisms in order to focus therapies on appropriate disease groups. A subset of ALS develop a devastating cognitive disease called frontotemporal dementia (FTD), and this ALS subgroup may have disease mechanisms distinct from ALS without FTD. Interestingly, these two diseases share pathological and genetic similarities, and it is unclear why patients with these same pathological and genetic features may develop such clinically distinct disorders. This project will take advantage of our extensive brain bank to identify pathological differences in the brains and spinal cords of patients dying with ALS, with or without dementia. In addition, we will examine the differences in proteins in these brains (proteomics) looking for patterns or “signatures” of disease that can differentiate ALS patients with and without dementia. Finally, we will test each patient for genetic mutations that may occur ALS and FTD as a further differentiating factor.

Augusta - Georgia Regents University

Lin Mei M.D./Ph.D.

RG Mechanisms of LRP4 autoantibodies in myasthenia gravis

$130,000.00	8/1/2014	7/31/2015	Year 3

Summary Myasthenia gravis (MG) is caused by autoantibodies against muscle nicotinic acetylcholine receptor (AChR) and MuSK, a receptor tyrosine kinase that is critical for agrin-induced AChR concentration at the neuromuscular junction (NMJ). However, some MG patients are negative for autoantibodies against AChR or MuSK. A better understanding of the pathogenic mechanisms of “seronegative” MG should have a major impact on diagnosis and treatment of these patients. In preliminary studies we found that sera of “seronegative” patients contained autoantibodies against LRP4, a receptor of agrin essential for NMJ formation. This result is exciting, but raises a critical question whether the LRP4 autoantibodies are pathogenic and if so, what the underlying mechanisms are. We will address these questions in this proposal. Results of the proposed research should contribute a better understanding of “seronegative” MG and development of novel diagnostic and therapeutic strategies for this devastating disease.

ILLINOIS

Champaign - The Board of Trustees of the University of Illinois at Urbana-Champaign

Steven C. Zimmerman Ph.D.

RG Discovery of New Therapeutic Agents for Myotonic Dystrophy Type 1 (DM1)

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Mytotonic dystrophy (DM1) is the most common form of muscular dystrophy with approximately 1 in 8000 people in North America afflicted by the disease. At the current time there is no cure for DM1 and remarkably no therapeutic agent to treat the disease, only drugs for symptomatic relief. The exciting finding that the disease originates and advances with a progressive expansion of a CTG sequence in the DMPK gene (chromosome 19) provides several targets for drug discovery. It is now generally accepted that the large expansions of the repeated CTG sequence of DNA, is transcribed into RNA and the RNA is toxic because it binds a key regulatory protein called muscleblind-like protein (MBNL). MBNL controls the correct expression of proteins that are important for a number of processes including relaxing muscles after contraction and insulin regulation. We recently identified a new, cell permeable ligand that inhibits MBNL binding to the toxic RNA. The goal of the proposed research is to further develop this and structurally related compounds, thereby developing more effective lead therapeutic agents. Our approach involves exploring the structural feature of the small molecule agent that are most important as well as developing a rapid way to assemble more potent agents using the RNA as a template to select optimum drug candidates. We propose to advance lead compounds from cellular assays to mouse models of DM1.

Chicago - Ann & Robert H. Lurie Children's Hospital of Chicago

Christine DiDonato PhD

RG SMN inductive therapy in mild SMA

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Spinal muscular atrophy (SMA) is caused by reduced levels of the survival motor neuron (SMN) protein. It is currently unknown how late in the disease process SMN inductive therapies can be beneficial in terms of either improving function or halting disease progression. This proposal focuses on answering that question by determining the latest time that SMN can be re-introduced after disease onset in milder forms of SMA and where it is required. We will specifically determine if therapies that only increase SMN within the nervous system can correct all deficits in milder forms of SMA. This research has important implications for SMA therapy development and the molecular mechanisms that contribute to disease.

Chicago - Illinois Institute of Technology

Nick Menhart Ph.D.

RG Biophysics of Exon Skipped Dystrophin Rods

$85,837.00	2/1/2014	7/31/2015	Year 3

Summary Most defects causing DMD delete a relatively small fraction of the dystrophin gene, but in a way that derails the process of turning this gene into dystrophin protein. In many cases, skipping over this damaged region with small molecule drugs called AONs restores the production of some dystrophin, which is expected to provide great clinical benefit and provide a potentially highly effective treatment. AON therapy essentially aims to convert DMD to the less mild condition, BMD which in many cases also has a fraction of the gene deleted, but in such a way that protein production is not derailed. However, the clinical severity of BMD is highly variable (in some cases with quite similar underlying defects), and so how complete an improvement might be expected is uncertain, and dependent on the exact fashion in which the defective region is skipped. For many DMD defects, alternative repairs are possible, skipping alternative exons and producing differently edited final proteins. Our previous work has shown such alternatives are sometimes of dramatically different properties. Unfortunately, the genetics of DMD are highly variable, with large number of defects known, and it is impossible extrapolate from these test cases to all defects. However, by protocols developed, will expand these tests beyond these simple test cases, to a wider range of defects that are being currently evaluated for AON (and other) therapies, and obtain data on which alternatives to pursue.

Chicago - The Board of Trustees of the University of Illinois - Chicago

Muthusamy Thiruppathi Ph.D

DG Defect in Immune Regulation in Myasthenia Gravis: Implications for Treatment.

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary Autoimmune myasthenia gravis (MG) is caused by a failure of immune regulation in which immune cells mistakenly target specific proteins on skeletal muscle. In other autoimmune disorders, a defect in the number or function of a specialized subset of immune cells, called regulatory T cells (Tregs) has been demonstrated. We have recently shown that Tregs from MG patients are present in normal numbers in the peripheral circulation but are poor immune suppressors. In the studies proposed in this application, we will thoroughly examine the nature of this immune defect in MG using blood cells collected from MG patients and healthy control subjects. Moreover, we will explore a strategy to enhance the function of these cells as a novel therapeutic approach in MG.

Chicago - The University of Chicago

JianRong Sheng Ph.D, M.D.

RG Immunomodulation of Experimental Autoimmune Myasthenia Gravis

$105,686.00	8/1/2014	7/31/2015	Year 3

Summary The symptoms of myasthenia gravis (MG) result from cells of the immune system attacking the body's own cells, namely the acetylcholine receptors of skeletal muscle. In MG, specific immune cells, B cells, produce antibodies (with the help of T cells) that bind to the muscle and produce muscle damage and weakness. Current treatments for MG suppress the immune system as a whole. Unfortunately, these treatments are not focused and cause widespread changes in immune function, increasing the risk for infections and malignancy. We have used a particular growth factor (GM-CSF) to induce a specialized type of regulatory immune cell (regulatory T cell) in mice with experimental MG, and have successfully suppressed MG in these mice. Our preliminary data also showed that GM-CSF not only induced regulatory T cell production but also expanded regulatory B cells in the mouse model of MG. Thus, it appears that this treatment leads to suppression of the autoreactive immune cells by inducing both regulatory T cells and regulatory B cells . Since B cells play a more direct role in MG, we now propose to examine methods of generating “regulatory B cells” using GM-CSF. We will further explore the potential of these cells as a treatment for MG. The information gained from these studies may help to develop a better treatment for human MG that is more focused, and potentially may eliminate the need for chronic immunosuppression.

INDIANA

West Lafayette - Purdue University

Shihuan Kuang Ph. D.

RG Targeting hypoxia signaling to improve the efficiency of myoblast transfer

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Satellite cells are muscle resident stem cells that mediate the regeneration of damaged skeletal muscles. Hence, myoblast transfer (MT), or transplantation of satellite cell derived myoblasts, represents a promising stem cell based therapy to treat degenerative muscle diseases such as Duchenne Muscular Dystrophy. However, the utility of this procedure has been limited due to the extremely low survival rate of transplanted cells. As myoblasts are typically cultured under ambient oxygen (O2) levels that are much higher than those within the skeletal muscle, especially injured and ischemic muscles, pre-treatment of cells to be transplanted with low O2 (hypoxia) should increase their survival in vivo and improve the efficiency of MT. Our group recently demonstrated for the first time that hypoxia conditioning indeed enhances the efficient of MT through activation of downstream signaling cascades. In this proposed study, we will investigate the role of HIF1a, a central mediator of hypoxia signaling, in satellite cell function in vivo. We will further define the optimal levels of O2 and patterns of hypoxia exposure that lead to maximal survival, proliferation, differentiation and homing of transplanted cells. Results from this study will increase our understanding of how O2 as an environmental factor affects satellite cell activity and lead to clinical applications that combining hypoxia conditioning to improve the efficiency of stem cell based therapy to treat muscular dystrophy.

IOWA

Iowa City - The University of Iowa

Kevin Peter Campbell PhD

RG Protein O-mannosylation: Classification of new players in muscular dystrophy

$125,000.00	8/1/2014	7/31/2015	Year 3

Summary Protein O-mannosylation is a rare type of post-translational protein modification in mammals, which when deficient can lead to progressive muscle wasting with potentially profound brain abnormalities. There is a critical need for better understanding of the enzymatic mechanism responsible for this modification to develop new treatment options for O-mannosylation deficient disease. Besides direct patient health benefits, identification of new players involved in protein O-mannosylation will open new avenues to understand O-mannosylation deficient muscular dystrophies.

Nivedita Jerath M.D.

CRTG Driving Ability in Patients with CMT 1A

$76,140.00	7/1/2014	6/30/2015	Year 1

$76,140.00	7/1/2015	6/30/2016	Year 2

Summary Charcot Marie Tooth 1A (CMT 1A) is a common inherited disorder of the nerves in the body. The disease can result in difficulties with strength and balance as well as in foot deformities such as high arches, hammer toes, and tight ankles. Because of these difficulties, the disease may affect driving, especially because driving requires quick responses at times such as slamming on the brakes or turning the steering wheel quickly. The following study tries to figure out if patients who have CMT 1A can drive normally or not. The study will have patients drive in a driving simulator, which involves a car located in a room in the basement of the hospital. Patients will drive in the car as if they are driving in real life and the whole experience is like playing a car video game with a big animated screen. There is also a special car that can video tape driving while patients are actually driving on the roads of Iowa City. If patients with CMT 1A do have driving difficulties, the results of the study will try to help patients with CMT1A drive better by ultimately creating devices that might help them use the steering wheel or brake pedal in a safer way.

Michael Shy M.D.

RIG North American Charcot-Marie-Tooth (CMT) Consortium

$147,951.00	1/1/2015	12/31/2015	Year 3

Summary The Inherited Neuropathy Consortium (INC) is an international consortium of centers funded by the MDA and NIH devoted towards developing treatments for and treating patients with inherited peripheral neuropathies known as Charcot Marie Tooth disease (CMT). Three thousand subjects are registered in various INC protocols that investigate how different types of CMT progress, develop outcome measures in children and adults to be used in clinical trial development, identify genetic changes that modify the severity of CMT, and identify new genetic causes of CMT. We also are training the next generation of researchers in CMT, developing standards of care for people with CMT, developing clinical trials and linking with National CMT programs throughout the world.

Michael Shy M.D.

RG Identification and Treatment of ER Stress in Patients with CMT1

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Charcot Marie Tooth (CMT) is the most common genetic neuromuscular disease. CMT1B is the second most common form that affects the myelin insulation . We have used data acquired from more than 100 patients with CMT1B to develop a hypothesis that we have used to develop treatment strategies for these patients. We have successfully tested this hypothesis in mouse models of CMT1B in work that was supported by the MDA. We wish to extend this work so that we can develop clinical trials in patients with CMT1B.

MARYLAND

Baltimore - Johns Hopkins University School of Medicine

Mohamed H. Farah PhD

RG Enhancing neuromuscular reinnervation by BACE1 inhibition

$125,000.00	2/1/2014	1/31/2015	Year 2
$125,000.00	2/1/2015	1/31/2016	Year 3

Summary This application tests an attractive potential therapeutic intervention for injured and diseased motor nerve. The overall goal of this application is to investigate whether capacity of motor nerve to regenerate after insult or disease can be enhanced to a degree that results in functional recovery. We will test whether drugs originally designed for Alzheimer's disease can bring out beneficial effect for motor nerve regeneration and restoration of neuromuscular function in preclinical animal models. We will test these drugs in early stage of motor neuron disease in a mouse model of Lou Gehring's disease.

Jeffrey D. Rothstein M.D., Ph.D.

RG ALS C9ORF72 iPS cells: Development of an antisense-based therapy and biomarker

$130,902.00	2/1/2014	1/31/2015	Year 2
$130,902.00	2/1/2015	1/31/2016	Year 3

Summary Understanding the pathophysiology and development of new therapeutics for amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's Disease) and dementias such as Alzheimer's and frontotemporal dementia has been an enormous challenge. The ability to actually have human cell lines that represent the natural disease by carrying hereditary gene mutations will provide unprecedented tools. In this application we propose to study molecular events that may contribute to the disease of a newly discovered common gene

mutation in ALS (C9ORF72) which is found in inherited (familial) as well as the common sporadic forms of ALS. We will employ ALS patient-derived human fibroblasts and convert them into adult induced pluripotent stem (iPS) cells as well as differentiated relevant central nervous system (CNS) cell types such as astroglia and motor neurons. These human cells will undergo a thorough analysis of their molecular genetic composition, which will then be compared to the genetic profile of human cells obtained from normal, healthy volunteers. Based on the differences we will design and develop a molecular therapeutic agent targeted at the specific mutation responsible for the disease. We will further develop a so called biomarker which will allow us to non-invasively monitor the efficacy of these novel drugs when given to patients. The use of these human cells may allow us to efficiently and quickly develop a drug therapy for C9ORF72 form of ALS.

Jeffrey D. Rothstein M.D., Ph.D.

RRG Robert Packard Center for ALS Research (Wings 2013) (Rothstein, Jeffrey)

$102,308.00	11/1/2014	10/31/2015	Year 1

Summary MDA funding received (as designated by Wings Over Wall Street) will be used to fund one (1) collaborative research project through the Robert Packard Center for ALS Research at Johns Hopkins. This project is affiliated with a Hopkins-based researcher who will participate in the Packard Center's collaborative process and whose proposal has been reviewed and approved by the Center's Scientific Advisory Board. Any additional funding required for this project beyond that awarded by MDA's Designated Grant will be covered by the Packard Center. Money received from this MDA Designated Grant will not be used to support Dr. Rothstein or his lab.

Charlotte Jane Sumner M.D.

RG Characterization of TRPV4 associated peripheral neuropathy in animal models

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Charcot Marie Tooth (CMT) disease is the most common inherited neurological disease. Mutations of TRPV4 cause both CMT 2C and distal SMA, which are both disease priorities for the MDA. Our long term goal is to determine how mutations in TRPV4 lead to peripheral nerve disease and to develop treatment for these diseases. We and others have shown that TRPV4 mutations cause increased channel activity, calcium influx, and cellular toxicity in transfected, cultured cells suggesting a gain-of-channel function. However, the mechanisms by which mutant TRPV4 causes peripheral nerve degeneration in vivo are unknown. In preliminary data, we have generated Drosophila and mice expressing mutant TRPV4, which now allow us to interrogate mutant TRPV4 activity in neurons as well as to investigate the consequences of mutant TRPV4 expression on peripheral nerve function in these two animal models. In this study, we will specifically evaluate whether mutant TRPV4 causes a gain of channel activity in neurons and whether this is associated with peripheral nerve distal axon degeneration.

Bethesda - Federation of American Societies for Experimental Biology

Grazia Isaya M.D., Ph.D.

SG FASEB Conference Mitochondrial Biogenesis & Dynamics in Health, Disease, Aging

$7,400.00	1/1/2015	5/31/2015	Year 1

Summary Growing evidence indicates that mitochondrial defects are directly and indirectly implicated in the pathophysiology of a broad range of conditions including metabolic disorders, neuromuscular disorders, adult-onset and age-associated neurodegenerative diseases and aging. The main goal of the 2015 FASEB Conference “Mitochondrial Biogenesis and Dynamics in Health, Disease and Aging” organized by G Isaya (Mayo Clinic) and L Pon (Columbia University) is to enable exchange of knowledge and promote collaborations between investigators who study basic aspects of mitochondria with translational investigators who study mitochondrial roles in human health and disease. These interactions will advance innovative interdisciplinary approaches to the study of mitochondria at the fundamental, translational and clinical levels. This Conference is relevant to MDA as 6 of its 8 sessions focus on aspects of mitochondria that represent potential therapeutic targets to restore/maintain mitochondrial integrity in neuromuscular disorders. Moreover, 2 additional sessions focus on new tools and new lines of research to interrogate mitochondrial function and its contribution to different pathologies, and develop novel strategies to correct primary and secondary mitochondrial deficits. We have lined up 33 outstanding mitochondrial experts and expect attendance by over 100 researchers from which we will select 16 additional speakers to give short talks on hot topics with emphasis on young investigators.

Rockville - ReveraGen BioPharma, Inc.

Eric Hoffman Ph.D.

MVP A Phase 1 Randomized, Placebo-controlled, Double Blind, Single Ascending and Multiple

Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmcokinetics and Pharmacodynamics of VBP15 in Healthy Adult Volunteers

$507,600.00	1/1/2015	5/1/2015	Year 2

Summary The goal of ReveraGen' s drug development program for DMD is to develop a dissociative glucocorticoid analogue that shows equal or greater efficacy than traditional glucocorticoids yet elicits fewer side effects. The company's focus has been on delta-9,11 dissociative analogues, specifically, C21 steroid analogues that lack glucocorticoid receptor mediated transcriptional activities but retain alternative signaling activities including transrepression of pro-inflammatory transcription factors (i.e. NF-?B) and membrane stabilization properties and do not require receptor-mediated gene transcriptional activity associated with deleterious side effects. Thus, the objective is to make an analogue as efficacious as traditional steroids, such as prednisone with less toxicity.

MASSACHUSETTS

Boston - Brigham and Women's Hospital, Inc.

Xin Wang Ph.D.

RG Identifying 2-Iodomelatonin and 8M-PDOT for ALS Therapy

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary We will evaluate novel agonists of melatonin receptor 1A (MT1) as potential drug candidates for ALS. We will characterize the neuroprotective signaling pathways associated with the agonist-MT1 axis. With support from an MDA grant, our preliminary results showed that N-Acetylserotonin (NAS) or melatonin delayed disease onset and mortality in ALS mice and inhibited cultured motoneuron death. Interestingly, two other MT1 agonists (2-iodomelatonin and 8M-PDOT) strongly protected motorneuronal cultures from cell death. What's more, our preliminary data in a small number of animals show that 2-iodomelatonin, in a very potent manner, significantly delayed disease onset and mortality in mSOD1G93A mice. This translational project aims to develop powerful MT1 agonist approaches for ALS therapy: 1) To conduct 2-iodomelatonin and 8M-PDOT trials before onset "preventively" and administer 2-iodomelatonin/8M-PDOT/NAS/melatonin at disease onset as "therapeutic treatment" in ALS mice; To measure their levels in blood, brain, spinal cord, and muscle of ALS animals by LC/mass spectrometric analysis. 2) To determine the additive effect of MT1 agonist combined with riluzole in cultured motoneurons and ALS mice. 3) To test the agonist-MT1 receptor axis activating PI3K-Akt-CREB and ERK/CREB signaling pathways and determine the effects of agonists in preventing neuronal cell death, neuropathological changes, SOD1 expression and aggregation, and proteasomal abnormality and autophagy dysfunction.

Boston - Children's Hospital Boston

Matthew Alexander Ph.D.

DG Role of miR-486 in the pathogenesis of Duchenne Muscular Dystrophy

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary The absence of dystrophin protein in DMD muscle results in dysregulated secondary signaling pathways which remain poorly understood. We have previously shown that a muscle-enriched microRNA, miR-486, is significantly reduced in human DMD biopsies. Our hypothesis is that overexpression of miR-486 in skeletal muscle will have a therapeutic effect in ameliorating some of the disease pathology associated with DMD. We have preliminary data demonstrating that miR-486 overexpression in mdx5cv (dystrophin mutant) mice can ameliorate some aspects of the disease progression. We will modulate the levels of miR-486 in muscle using transgenic mice on the mdx5cv background to determine how miR-486 overexpression ameliorates the mdx phenotype. We will transiently overexpress miR-486 using adeno-associated virus (AAV) intramuscular injections to determine if miR-486 overexpression can be beneficial to mdx muscle. Our main goal of understanding the therapeutic potential of miR-486 overexpression in dystrophic muscle will be studied via the following specific aims: 1) To analyze the therapeutic potential of miR-486 overexpression in vivo, using transgenic and AAV expression of miR-486 in the normal and dystrophin-deficient mouse muscle. 2) To analyze the miR-486 null mouse and to identify the effects of miR-486 deficiency on muscle function.

Da-Zhi Wang Ph.D.

RG The miR-155-MEF2A axis in muscular dystrophy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Defective muscle regeneration and function is associated with neuromuscular diseases, including muscular dystrophies. However, the molecular targets that regulate skeletal muscle development, function and regeneration remain poorly defined. Our lab has previously demonstrated that muscle-specifically expressed miRNAs, including miR-1, miR-133 and miR-206, modulated muscle cell and satellite cell proliferation, differentiation and muscle regeneration. Most recently, we found that the expression and function of MEF2A, a member of the MEF2 family of myogenic enhancer factors, was regulated by miR-155. Interestingly, the expression level of miR-155 was increased in the skeletal muscle of mdx mice, an animal model for human Deuschenne Muscular Dystrophy. We further showed that overexpression of miR-155 inhibits myoblast differentiation in myoblast cell line. Most importantly, we found that mice with genetic deletion of miR-155 displayed better muscle function and regeneration. The overall goal of this study is to uncover the involvement of the miR-155-MEF2A axis in muscular dystrophy. We design three aims to achieve this goal, using a mouse model of human muscular dystrophy. Together, our studies will define the biological function of miR-155 in muscle function and regeneration. miR-155 could become a novel therapeutic target to treat muscular dystrophy.

Boston - Harvard University School of Public Health

Marc Weisskopf Ph.D., Sc.D.

RG Population-based Epidemiology Study of ALS in a Representative Sample of the US

$100,538.00 8/1/2014 7/31/2015 Year 3

Summary We still have a very limited understanding of fundamental aspects of the distribution of amyotrophic lateral sclerosis (ALS) in the US, such as the distribution by race/ethnicity and socioeconomic factors. Related to this, the progress in the identification of etiologic risk factors for ALS has been quite slow. The lack of very large cohort studies that are representative of the population, and in which relevant data is collected prospectively, prior to ALS is an important contributor to these limitations. In this project, we will take advantage of a unique data set that includes almost 2.4 million US men and women, is representative of the US population, has collected data prospectively, and has been followed for cause of death from which we can identify ALS cases, 713 of which have already been identified, with more anticipated before this project is completed. These data provide us a unique opportunity, with which we will determine - with by far the strongest data to date - the distribution of ALS by race/ethnicity and socioeconomic factors. We will also be able to determine the relevance of military service to ALS, and explore ALS risks by occupation and occupational lead exposure - key factors that would provide important clues to disease pathogenesis and suggest future avenues for research that have a higher likelihood of identifying specific etiologic agents for the development of ALS.

Boston - Massachusetts General Hospital (The General Hospital Corp.)

Vera Fridman M.D.

CRTG Effect of L-serine supplementation on clinical progression in HSAN1

$90,000.00 9/16/2014 9/15/2015 Year 2

Summary Hereditary sensory and autonomic neuropathy type I (HSAN1) is a rare genetic neuropathy that causes severe numbness, weakness and ulceration of the feet and hands. Recently, two abnormal lipids were identified in the blood of both humans and mice with HSAN1. It has been shown that these lipids can be reduced by administering the amino acid Serine to both humans and mice with HSAN1, and that mice that are given Serine have better motor and sensory function. The current study aims to address the effect of Serine on the symptoms of patients with HSAN1 in order to assess whether this may be an effective therapy for the neuropathy.

Thurman Wheeler M.D.

RG Progressive myopathy and therapeutic development in myotonic dystrophy type 1

$132,000.00 8/1/2014 7/31/2015 Year 3

Summary Myotonic dystrophy (dystrophia myotonica; DM) is the most common muscular dystrophy in adults, affecting approximately 1 in 7,500 people. At present, there is no cure, and no treatment alters the disease course. The most debilitating features of DM type 1 (DM1) are progressive muscle weakness and wasting. The mechanism responsible for progressive muscle degeneration in human DM1 is unknown. Although the disease mechanism in muscle tissue has been well characterized in young DM1 mice, they have a muscular dystrophy that is mild relative to human DM1. By contrast, the muscle degeneration in aged DM1 mice is substantially worse than in young mice, approaching the severity in human DM1. We have developed novel therapies that correct most aspects of the muscle disease in young DM1 mice. However, it is unclear whether these therapeutic agents will demonstrate similar safety and efficacy in aged DM1 mice that have advanced muscular dystrophy. In this project we will use a DM1 mouse model to characterize the disease mechanism in aged DM1 muscle. Goals include, 1) determine why progressive muscle wasting occurs in DM1; 2) test newly developed therapeutic agents in aged DM1 mice, which may be more predictive of safety

and therapeutic response in human DM1 individuals.

Boston - Trustees of Boston University

Jeffrey Boone Miller PhD

RG CMD & LGMD therapeutic targets: Studies with patients' myogenic cells

$114,620.00	2/1/2014	7/31/2015	Year 3

Summary Our studies are designed to identify new therapeutic strategies for a group of rare congenital and limb-girdle muscular dystrophies for which there currently are no effective ameliorative treatments. We have identified a molecular pathway that is abnormally activated within diseased muscle cells and thereby causes muscle cell death. Our goals in this project are to (i) further identify the mechanisms by which this muscle cell death occurs and (ii) develop therapeutic strategies that will ameliorate disease by preventing the abnormal cell death.

Concord - Valerion Therapeutics, Inc.

Dustin Armstrong Ph.D.

MVP Muscle Targeted Myotubularin 1 for Treatment of Congenital Myotubular Myopathy

$661,839.00	8/1/2014	2/28/2015	Year 3

Summary A. Specific Aims, Rationale and Significance X-Linked Centronucleolar Myopathy (XLCNM), also referred to as Myotubular Myopathy (MTM) is a rare Xlinked congenital myopathy with an estimated incidence of 1:50,000 live-born males. The myotubularin gene (MTM1), mutated in XLCNM, encodes a protein tyrosine phosphatase (1-6). Mice possessing a targeted inactivation of the MTM1 enzyme (MTM1 KO) show restricted development of muscle mass due to small myofibers, muscle weakness, respiratory collapse and death at a median age of 6 weeks (Figures 1 and 2, (7)). MTM1 is ubiquitously expressed yet its absence in skeletal muscle solely accounts for the pathophysiology of XLCNM (4-9). A therapeutic strategy with the greatest clinical benefit for XLCNM patients would likely require restoration of MTM1 function to skeletal muscle either through gene, stem cell or recombinant protein delivery. Of these methods, recombinant protein replacement is an established treatment for various enzyme deficiencies

MICHIGAN

Ann Arbor - The Regents of the University of Michigan

Anthony Antonellis Ph.D.

RG Correcting the molecular defect of CMT-associated tRNA synthetase mutations

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Charcot-Marie-Tooth (CMT) disease is a heterogeneous class of disorders characterized by progressive muscle weakness and loss of sensation in the hands and feet. Currently, there is no cure for CMT disease. Importantly, many different genes have been implicated in CMT disease making it difficult for efficient therapeutic design. The human genome contains 37 tRNA synthetase (ARS) genes that encode a class of enzymes with similar functions in producing cellular proteins. To date, six of these 37 ARS genes have been implicated in CMT disease, and we predict that more ARS genes will be implicated in CMT disease in the future. Disease-associated ARS mutations impair the primary function of the enzyme suggesting that improving this function will be a relevant therapeutic strategy for patients with CMT disease. To address this, we will: (1) Systematically link impaired ARS function with CMT disease pathogenesis; and (2) Demonstrate that restoring ARS function will improve CMT disease characteristics. These efforts will have direct implications for developing therapies to treat the many patients with CMT caused by mutations in a large class of human genes.

Andrew Lieberman M.D., Ph.D.

RG Allosteric activators of Hsp70 to treat spinobulbar muscular atrophy

$34,375.00	8/1/2014	7/31/2015	Year 3

Summary Spinobulbar muscular atrophy (SBMA) is an inherited degenerative disorder of lower motor neurons that is caused by a CAG/glutamine tract expansion in the androgen receptor (AR) gene. The mutant protein causes testosterone-dependent toxicity that results in muscle weakness and atrophy in men. Prior work has established that the mutant AR protein is the cause of this toxicity, suggesting that strategies to enhance its degradation should diminish disease severity. Therefore, we sought to understand this process, and found that AR degradation is tightly controlled by a cellular machinery consisting of the heat shock protein 70 (Hsp70). We propose that stabilizing Hsp70 in a conformation that binds the mutant AR with high affinity will

promote its degradation. We will test this idea both genetically, using an Hsp70 interacting protein that stabilizes Hsp70 in its high affinity binding state, and pharmacologically, using a novel small molecule that we recently identified which functions similarly. We will also test a small molecule that activates Hsp70's binding to the mutant AR in SBMA mice. We hypothesize that promoting Hsp70 binding to the mutant AR will increase its degradation and alleviate toxicity in SBMA models. It is our expectation that this work will help define a new therapeutic approach to SBMA and other protein aggregation disorders where degradation of the mutant protein is controlled by Hsp70.

Daniel E Michele Ph.D.

RG Reversing nitric oxide synthase dysfunction in muscular dystrophy

$121,655.00 8/1/2014 7/31/2015 Year 3

Summary Muscular dystrophies are characterized by muscles that are weak, sensitive to injury, and fatigue rapidly during normal muscle activity. Recent work has focused on the role of loss of function of an enzyme nitric oxide synthase (nNOS) in muscle causing fatigue in muscular dystrophy. nNOS produces nitric oxide, which is required for maintaining increased blood flow to muscle during activity. Very little is known about how nNOS is regulated in muscle. Although nNOS localization to the cell membrane is disrupted in Duchenne muscular dystrophy, the broad disruption of nNOS localization in other muscular dystrophies with normal dystrophin expression, raises considerable questions about what causes NOS dysfunction in dystrophic muscle. An important regulator of nitric oxide synthase activity in whole animals is modified forms of the amino acid arginine, that circulate in the bloodstream and inhibit nitric oxide synthase. Our preliminary data show that methylated arginines are markedly elevated in serum of dystrophic mice, are acutely increased in result to direct skeletal muscle injury, and experimental elevation of methylated arginines is sufficient to reduce running exercise capacity in normal animals. This project will test if methylated arginines cause muscle fatigue, and test directly if reducing methylated arginines in dystrophic animals, reduces muscle fatigue/weakness and slows development of cardiomyopathy, and provides therapeutic benefit to dystrophic animals.

MINNESOTA

Minneapolis - Regents of the University of Minnesota - Twin Cities

Atsushi Asakura Ph.D.

RG Angiogenesis-based therapy for muscular dystrophy

$121,840.00 8/1/2014 7/31/2015 Year 3

Summary Duchenne Muscular Dystrophy (DMD) is caused by mutations in the dystrophin gene, which functions to maintain muscle fiber structure, preventing it from being damaged by muscle contraction. Current treatment focuses on prolonging survival and improving quality of life. Recent work has demonstrated the involvement of dystrophin in blood flow regulation, which might be disturbed in DMD, possibly furthering muscle damage. However, the importance of angiogenesis in DMD treatment has not yet been well addressed. It may be possible to reduce muscle fiber damage by using angiogenic factors to increase the number of blood vessels and observe the resultant effects on the muscular dystrophy phenotype. We hope that these angiogenic factors will improve the development of new therapies for DMD via increased vascular density in blood starved dystrophic muscles.

Rita R. Perlingeiro Ph.D.

RG DMD IPS CELLS: GENETIC CORRECTION AND MUSCLE REGENERATION

$130,000.00 8/1/2014 7/31/2015 Year 3

Summary There has been tremendous excitement for the therapeutic potential of iPS cells in treating genetic diseases. This application builds on our successful proof-of-principle studies for DMD performed with mouse wild-type and dystrophic iPS cells as well as control (healthy) human iPS cells, which demonstrate equivalent functional myogenic engraftment to that observed with their embryonic counterparts following their transplantation into dystrophic mice. Our goal now is to apply this technology to iPS cells obtained from patients with Duchenne Muscular Dystrophy by establishing methods to genetically correct the disease, and to evaluate the regenerative potential of resulting genetically corrected iPS cells in dystrophic mice.

MISSOURI

Columbia - The Curators of the University of Missouri

Dongsheng Duan PhD

RIG A canine tissue bank for Duchenne muscular dystrophy study

$84,600.00 5/1/2014 4/30/2015 Year 1

$84,600.00 5/1/2015 4/30/2016 Year 2

Summary Lay Summary Animal models are necessary to understand the molecular process of Duchenne muscular dystrophy (DMD), a fatal disease caused by dystrophin gene mutation. More importantly, animal models are indispensable in testing novel therapies. Dystrophic dogs and human patients share similar disease features. It is expected that results generated from dogs are more likely to translate to DMD patients. In this application, we will set up a national dog tissue bank to provide DMD investigators with access to normal and dystrophic dog tissues. Successful establishment of this tissue bank will accelerate the tempo of DMD research in numerous directions.

Christian Lorson PhD

RG Evaluation of SMA pathways with scAAV9 vectors

$127,194.00	2/1/2014	7/31/2015	Year 3

Summary Spinal Muscular Atrophy (SMA) is a devastating neurodegenerative disease that is the leading genetic cause of infantile death. Recently, results in animal models of SMA have shown that a gene therapy approach can profoundly improve, and in some instances, nearly correct the SMA phenotype. The vectors used in these experiments is called scAAV9. Based upon this work, we plan to explore how additional transgenes may impact the SMA phenotype when expressed from a scAAV9 vector. This work has the potential to shed light upon the functional deficit that leads to SMA development as well as identify additional targets for therapeutic development.

St. Louis - St. Louis College of Pharmacy

Martha Bhattacharya Ph.D.

DG Molecular Mechanisms of Peripheral Axonal Degeneration

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary In neuromuscular diseases where motoneuron dysfunction is the primary cause of disability, such as amyotropic lateral sclerosis (ALS) and Charcot-Marie-Tooth (CMT) disease, axonal degeneration is a unifying pathological hallmark of disease progression. Axonal degeneration occurs via an active molecular cascade that results in swelling, fragmentation, and eventual loss of axons and neuromuscular synapses. We have developed a model of axonal degeneration in the genetically tractable fruit fly Drosophila melanogaster. Using this model, we have performed a screen to identify necessary components of the axonal degeneration cascade and have demonstrated that a number of these genes also have roles in mammalian axonal degeneration. To take these findings closer to clinical application, we must understand the pathways controlled by these molecules to identify steps amenable to interference. One gene we have identified is a putative G-protein coupled receptor (GPCR); these receptors are highly desirable drug targets. Another is a protein kinase for which specific inhibitors are available. For the GPCR, we will determine its signaling mechanism in mammalian neurons and assay its ability to protect neuromuscular synapses after injury. For the kinase, we will examine the effects of loss of this protein in vivo on mouse axons and synapses. Finally, we will prioritize other newly discovered proteins using cellular assays to assess their therapeutic potential.

St. Louis - Washington University in St.Louis

Anne M Connolly M.D.

HCTG Phase 2 Historically Controlled Trial of Corticosteroids in Young Boys with DMD

$136,858.00	1/1/2015	12/31/2015	Year 3
$.00	1/1/2016	4/30/2016	Year 4

Summary While it has been known for many years that corticosteroid use benefits boys with DMD, most clinicians do not consider treating until after age 3 or 4 years of age. The primary reason for the delay is that daily corticosteroid use has many side effects including short stature, obesity, and osteoporosis. A recent randomized blinded study of weekend oral corticosteroid use over one year showed equal improvement in strength with fewer side effects, particularly as related to growth and cushingoid changes. We will test the efficacy of oral weekend corticosteroid use in infants and young boys with DMD who are under age 30 months. We have demonstrated that the Bayley-III Scales of Infant development shows that infants and young boys in this age group who are untreated decline in abilities when compared to their peers. Furthermore, the North Star Ambulatory Assessment which scores the ability to walk, run, and take steps shows scores that are lower than typically developing boys. Here, in this Phase 2 historically controlled trial, we will use these two measures and treat boys at five MDA-DMD centers.

Jeffrey D. Milbrandt MD, PhD

RG Manipulating Schwann cell metabolism to treat peripheral neuropathy

$119,122.00	8/1/2014	7/31/2015	Year 3

Summary Neuropathies and neuromuscular diseases like CMT, Friedreich's ataxia and ALS appear to be linked to poor mitochondrial function, which is the key energy producer of the cell. We found that mutant mice with mitochondrial deficits in Schwann cells, a type of glial cell that supports neuronal function and survival, develop progressive neuropathy that mimics key components of human neuromuscular disease. We plan to investigate how abnormal Schwann cell metabolism causes nerve damage in patients with neuropathy. Moreover, we will test whether specific drugs can restore normal nerve function in mouse neuropathy models.

Timothy M Miller M.D.,Ph.D

HCTG Natural History Study of Familial ALS

$59,500.00	1/1/2014	3/31/2015	Year 3

Summary We are currently developing therapies for familial ALS. In order to better understand how these therapies are working and to design future clinical trials, we need more information about subjects with familial ALS. Our study is designed to retrospectively gather information on disease progression and survival in patients with familial ALS.

Kelly Renee Monk Ph.D.

RG Control of myelination by G protein-coupled receptor signaling

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Myelin is the fatty insulation that covers nerves and allows for the nervous system to function properly. In the peripheral nervous system (PNS), Schwann cells make myelin. In many peripheral neuropathies, myelin is damaged or malformed, causing debilitating symptoms. Unfortunately, current treatments are limited, and there is a pressing need to develop therapies for PNS diseases. A protein called Gpr126 is required for PNS myelination. In mouse and zebrafish Gpr126 mutants, Schwann cells cannot make myelin. Gpr126 belongs to a class of proteins called G protein-coupled receptors (GPCRs). GPCRs are excellent therapeutic targets, representing at least one-third of all available prescription drugs. Gpr126 therefore represents a new drug target in patients with PNS disease. Before Gpr126 can be considered as a drug target in humans, however, we must learn more about how it functions to control myelination. Additionally, it is important to know whether other GPCRs are important for myelination. To this end, we have discovered that the related GPCR, Gpr56, is also important for Schwann cell myelination in the PNS. In the proposed project, we will define the function of Gpr56 in Schwann cell development, myelination, and myelin maintenance. We will also determine small molecules and proteins that can activate Gpr126. Importantly, Gpr126-activating compounds and proteins can represent new drug targets in PNS disease.

NEVADA

Reno - Board of Regents, NSHE, obo University of Nevada, Reno

Dean J. Burkin Ph.D.

RG Laminin-111 protein therapy for Duchenne Muscular Dystrophy

$102,676.00	8/1/2014	7/31/2015	Year 3

Summary We have recently shown that laminin-111 protein therapy can prevent muscle disease in the mdx mouse model of Duchenne muscular dystrophy. At the time of diagnosis, Duchenne patients have already developed significant muscle disease and it is unclear if laminin-111 protein therapy is effective at preventing disease progression after it has already started. To translate the above exciting result into a therapy for Duchenne patients, we will determine if laminin-111 protein therapy can prevent muscle pathology after disease onset in mouse and GRMD dog models of Duchenne muscular dystrophy. Results from this study will pave the way for developing laminin-111 as novel therapeutic for DMD.

Ryan David Wuebbles Ph.D.

DG Laminin-alpha1 fragment and peptide therapy for Duchenne Muscular Dystrophy

$60,000.00	8/1/2014	7/31/2015	Year 3

Summary Although there is currently no effective treatment or cure for DMD, several promising therapeutics are currently being investigated. One of these is an extracellular matrix protein called Laminin-111, the embryonic paralogue to Laminin-211, which both interact with the dystrophin-associated glycoprotein complex (DGC) and alpha7beta1 Integrin. Within the mdx mouse model of DMD, the introduction of Laminin-111 leads to the prevention of muscle pathology and reduced exercise-induced muscle injury. These changes were likely brought about through the increased levels of both Utrophin and alpha7 Integrin proteins. However, the production of laminin-111 protein for the use as a therapy for DMD is difficult due to the size

of the heterotrimeric protein of over 900 kDa. These exciting results will be more quickly and easily brought into therapeutic use if a smaller part of the Laminin-alpha1 protein or peptide is capable of reproducing the effects of the entire laminin-111 protein complex. Here, we propose to determine if part of the Laminin-alpha1 protein is capable of producing the therapeutic effects of the entire complex. The results of this study could provide a novel protein therapy for DMD which would be more quickly and easily produced than that of the entire complex.

NEW JERSEY

Bridgewater - SANOFI-AVENTIS U.S. INC

Christopher Penton Ph.D.

B2I Identification of Therapeutics that Improve Skeletal Muscle Regeneration and Ameliorate Skeletal Muscle Atrophy

$60,000.00	6/1/2014	5/31/2015	Year 2
$60,000.00	6/1/2015	5/31/2016	Year 3

Summary We hypothesize that there are common cell signaling events between muscle satellite cells and fibroblastic-adipogenic progenitors (FAPs) that block the self-renewal of satellite cells and enhances the differentiation FABs into adipocytes and fibroblasts generating muscle fibrosis and a reduction of muscle function. This proposal will employ primary cell-based assays developed during the project to identify compounds that block the targets implicated in the disease pathology and potentially enhance muscle regeneration. Animal models of muscular dystrophy will be employed to evaluate muscle performance in response to drug treatment. The overall objective of this approach will be to identify drug candidates that improve skeletal muscle regeneration in muscle dystrophies4-6 and injury in order to offer innovative, therapeutic solutions to muscular dystrophy patients.

NEW MEXICO

Albuquerque - University of New Mexico HSC

Sarah Youssof M.D.

CRTG Outcome Measures in Oculopharyngeal Muscular Dystrophy

$90,000.00	7/1/2014	9/30/2015	Year 2

Summary Oculopharyngeal muscular dystrophy (OPMD) is a progressive, adult-onset, incurable muscle disease that leads to devastating inability to swallow and can cause disabling limb muscle weakness. Nearly a century after the first description of the syndrome, therapies that halt or slow muscle degeneration in OPMD do not exist. While the gene mutation is known, animal models have been developed, and several agents have shown promise in slowing disease progression in preclinical studies, there is a dearth of clinical trials for OPMD. A critical barrier to the pursuit of clinical trials is the lack of established outcome measures that can capture disease progression and treatment effects. The long-term goal of our research is to conduct clinical trials for OPMD incorporating validated outcome measures that reflect endpoints meaningful to patients. The overall objective of this application is to explore the performance of a set of outcome measures for measurement of OPMD disease severity and to investigate the patients' perspectives on the impact of disease. Since the largest cluster of OPMD in the United States occurs among Hispanic New Mexicans, UNM Health Sciences Center is the optimal location to conduct this research.

NEW YORK

Albany - Research Foundation of SUNY - University at Albany

Li Niu Ph.D.

RG Characterization of Chemically Modified Aptamers as New ALS Drug Candidates

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Excessive activation of the a-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtype of ionotropic glutamate receptors is an important pathogenic mechanism for ALS. Finding inhibitors to control the excessive receptor activity has been a long-pursued strategy for developing ALS drugs. We previously showed that nanomolar affinity RNA inhibitors or RNA aptamers selectively targeting AMPA receptors can be identified. These aptamers are superior to traditional, small-molecule inhibitors, because these traditional inhibitors are organic compounds and generally have poor water solubility, low affinity and cross activity. However, unmodified, these RNA aptamers are limited in therapeutic applications in vivo by their inherent sensitivity towards ribonucleases, the enzymes that catalyze RNA degradation. In contrast, chemical modifications of RNA molecules can turn them into ribonuclease-resistant or biostable aptamers. Thus, making biostable aptamers is the first step to translate these powerful AMPA receptor aptamers into clinically

useful drugs. Thus far, we have successfully developed several high-affinity, chemically modified aptamers for AMPA receptors. The goal of this proposal is to characterize these chemically modified RNA aptamers for their neuroprotective effectiveness on glutamate-induced neurotoxicity in ALS cellular and animal models. These studies are key preclinical experiments to advance these RNA inhibitors as a new ALS drug.

Bronx - Albert Einstein College of Medicine of Yeshiva University

Morayma Reyes M.D., Ph.D.

RG Role of PDGF Receptor alpha signaling in DMD cardiac fibrosis

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary We propose to study the effects of blocking PDGFRa signaling in ameliorating cardiac fibrosis in the mdx model of Duchenne muscular dystrophy (DMD) using Crenolanib, a potent PDGFRa inhibitor. Crenolanib is a new investigational oral drug currently in Phase II clinical trials to treat several cancers. Thus if these clinical trials prove safety and efficacy of Crenolanib use in children, then the studies proposed herein are the foundation of preclinical studies for the use Crenolanib to ameliorate fibrosis in DMD patients.

Brooklyn - The Research Foundation of SUNY on behalf of SUNY Downstate Medical Center

Charles K Abrams M.D., Ph.D.

RG Mechanisms of CNS Disease in X-Linked CMT

$134,764.00	2/1/2014	7/31/2015	Year 3

Summary Vertebrate gap junctions, composed of connexin proteins, form pathways between apposed cells; they allow for the diffusion of small molecules and ions. Over 300 mutations in the gene for connexin 32 have been linked to the inherited peripheral neuropathy CMT1X (X-linked Charcot-Marie-Tooth disease). CMT1X is unusual in that in addition to peripheral nervous system (PNS) dysfunction, many patients develop central nervous system (CNS) signs and/or symptoms. This is presumably the result of connexin 32 being expressed in both ensheathing cells of the PNS (Schwann cells) and ensheathing cells of the CNS (oligodendrocytes). This project will generate new understanding of how mutations in the gene for a gap junction protein, connexin 32, may lead to CNS signs and symptoms in CMTX. The hypothesis driving this project is that mutations in connexin 32 cause CNS dysfunction by interacting with a related CNS protein, connexin 47, to reduce the oligodendrocytes ability to provide a diffusion pathway for potassium, which builds up during neural activity. Our findings should have important implications for the development of strategies to minimize the impact of these mutations on both CNS and PNS manifestations of CMTX.

Buffalo - The Research Foundation of State University of New York on behalf of University at Buffalo (SUNY @ Buffalo)

Elisabetta Babetto Ph.D.

DG Phr1 as a novel regulator of axon integrity in Charcot-Marie-Tooth diseases

$50,760.00	5/1/2014	4/30/2015	Year 1
$50,760.00	5/1/2015	4/30/2016	Year 2
$50,760.00	5/1/2016	4/30/2017	Year 3

Summary The degeneration of the long axons in patients with Charcot-Marie-Tooth and other peripheral neuropathies causes symptoms such as muscle weakness. Thus it is important to understand the regulation of axon degeneration in order to slow it. This is especially compelling in genetic neuropathies when a diagnosis can already be achieved in asymptomatic patients. Widely used as a model, nerve degeneration after experimental injury is a regulated process in which molecular components in axons, associated glia, immune and other cells orchestrate a cascade of events that leads to fast disintegration of the distal nerve stump and subsequent nerve remodeling. We recently identified a protein which is a key molecular player of this process. Its inactivation strongly delays axon loss after mechanical injury or application of a chemotherapy drug. We will test if manipulation of the identified pathway can ameliorate axonopathy in two mechanistically distinct CMT mouse models. Moreover, because our data show a reduced protective effect if neurons are isolated from their ensheathing glia, we propose an additional protective role of this protein in these glia. Thus, we speculate about differential therapeutic benefits between CMT models in which axonal health is impaired as a consequence of glial abnormalities and models in which axonal health is intrinsically hampered. These experiments will improve our understanding of axonopathy and have the potential for novel treatments in CMT patients.

Buffalo - The State University of New York at Buffalo

Bogdan Karl Beirowski M.D., Ph.D.

DG Modeling axon loss in CMT by disruption of Schwann cell metabolic regulation

$60,000.00	8/1/2014	7/31/2015	Year 3

Summary Degeneration of long axons within peripheral nerves is a hallmark of Charcot-Marie-Tooth (CMT) diseases and the more severe forms of Dejerine-Sottas syndrome. This results in progressive muscle weakness and sensory deficits. Surprisingly, in many CMT diseases the primary molecular defect and dysfunction localizes to Schwann cells (SCs), a type of insulating cell that wraps axons with a multilayered membrane known as myelin. It is poorly understood how dysfunction in SCs results in axon loss. Previous work suggested that removal of defective myelin ('demyelination') causes axon damage due to inflammation in some models of CMT. However, there is no evidence for general applicability of this model, especially to CMT types that are not associated with overt demylination. This led to the hypothesis that abolished support by failed delivery of small metabolites from SCs into axons could explain the degenerative phenotype. To test this we developed novel mouse mutants in which key metabolic regulators are blocked exclusively in SCs. Strikingly, our data demonstrate age-dependent axonal demise in peripheral nerves from these mutants, but no demyelination. Here we will characterize alterations in mutant SCs employing innovative profiling technologies to identify metabolic signatures that contribute to the disrupted support of axons. If specific metabolic lesions can be identified in SCs, treatment by replacing enzymes or missing substrates becomes a realistic goal in axonopathies.

Ithaca - Cornell University

Fenghua Hu Ph.D.

RG Role of Ubiquitination in TDP-43 aggregation and clearance

$120,000.00	2/1/2014	1/31/2015	Year 2
$120,000.00	2/1/2015	1/31/2016	Year 3

Summary Aggregation of a protein called TDP-43 has been found in Amyotrophic Lateral Sclerosis (ALS), Frontal Temporal Lobar Degeneration and many other neurodegenerative diseases. Mutations in the TDP-43 gene are also found in a subset of ALS patients, suggesting that misbehavior of TDP-43 protein could cause neurodegeneration. TDP-43 found in the protein aggregates were often cleaved to generate aggregation prone C-terminal fragments. Furthermore, TDP-43 aggregates are often modified by ubiquitination, a process that add a small molecule ubiquitin to protein. However, the role of TDP-43 C-terminal fragments and ubiquitination in disease progression is still not clear. In the proposed project, we will first establish a zebrafish model to study TDP-43 C-terminal fragment induced toxicity and neurodegeneration. Next, we will determine the function of ubiquitination and ubiquitin binding proteins in TDP-43 aggregation and clearance. Our proposed studies will provide valuable insights into the mechanisms involved in TDP-43 aggregate formation and clearance as well as its toxicity in neurons.

New York - Columbia University Medical Center

Veronica Hinton Ph.D.

RG Executive Functions in Boys with Dystrophinopathy

$132,532.00	8/1/2014	7/31/2015	Year 3

Summary Children with dystrophinopathies are at risk for having cognitive and behavioral deficits in addition to muscle weakness. Our work has concentrated on studying these deficits in depth. We have documented that the selective verbal immediate memory deficits observed in children with dystrophinopathy are related to poorer academic achievement and may also be associated with behavioral problems. We now plan to continue and expand the study of cognitive skill development in boys with dystrophinopathy by focusing in detail on executive functions. Our goals are to examine executive skills in depth among a large sample of children with dystrophinpathy and examine the interplay of executive skills on “real life” outcomes of academic skill acquisition, peer relationships and behavioral adjustment. Additionally, we will also build on an existing cohort of 47 boys diagnosed with dystrophinopathy who will be assessed approximately 6.5 years after their parents completed an early measure describing their executive functions. This unique group will allow us to test whether the early rating scale may be predictive of later outcome, and as such useful as a clinical screen to determine children who may be at greatest risk for academic and social problems. We will examine the complex relationships among early executive function deficits and later academic achievement and psychosocial adjustment.

Michio Hirano M.D.

RG Molecular bypass therapy for TK2 deficiency

$132,844.00	8/1/2014	7/31/2015	Year 3

Summary Thymidine kinase 2 (TK2) deficiency is a rare genetic neuromuscular disease that typically begins in infancy and is fatal in childhood but can also manifest as adult-onset progressive external ophthalmoplegia. We have generated a mouse model with severely decreased Tk2 activity and reductions in its products. In preliminary studies, administration of compounds to bypass the defective Tk2 enzyme slowed the progression of the

disease and extended the lifespans of the mutant mice. Moreover, we demonstrated that the compound is able to penetrate into tissues including the brain. We propose to characterize the cause of the neuromuscular weakness and to optimize long-term treatment in the mutant mice. If we are successful, our studies may lead to a significant therapy for human TK2 deficiency and related diseases.

Ronald K. Liem Ph.D.

RG Characterization of a new mouse model for CMT2E

$106,088.00	2/1/2014	1/31/2015	Year 2
$106,088.00	2/1/2015	1/31/2016	Year 3

Summary This proposal seeks to characterize a new mouse model for Charcot-Marie-Tooth (CMT) type 2E that will help decipher pathogenic mechanisms. CMT is the most common hereditary neuropathy with a prevalence of 1 in 2500 worldwide. There are mutations in both myelin genes and neuronal genes that cause CMT. Mutations in the neurofilament light chain, the major component of intermediate filaments in the nervous system, cause a particular subtype of CTM called CMT2E. These mutations are dominant and the age of onset and severity of the disease is variable depending on the mutation. Based on clinical descriptions, we have chosen to study one particular mutation with an early age of onset and relatively severe symptoms. We generated a mouse model for CMT2E by knocking-in this particular mutation. The mutant mouse will therefore have one defective copy of the gene and one normal one similar to the human patients. The mutant mouse recapitulates the disease as found in humans with this mutation, including early onset of symptoms, motor defects, as well as hearing defects. This mutant mouse will therefore provide us with a model that will allow us to study the progression of the disease at a level that is not possible in humans. We expect that the mouse model will also be useful for testing therapeutic compounds when they become available, as well as to study the mechanisms by which the neurodegeneration occurs.

Hiroshi Mitsumoto MD

RRG 2014 Wings Over Wall Street Research Projects Proposal

$102,312.00	9/1/2014	8/31/2015	Year 1

Summary With the MDA Wings Over Wall Street fund, we will expand biostatistical analyses of the large baseline dataset of the ALS COSMOS study. Furthermore, we will establish a telephone-based cognitive screening test, which will be used for the first time ever in an epidemiologic study. With the establishment of this test, we will recruit a large number of patients from all 50 US States through the National ALS Registry.

Umrao R. Monani Ph.D.

RG Elucidating the role of the SMN protein in the developing neuromuscular system

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Although it is apparent that motor neurons are amongst the first cells to be affected by reduced SMN, the spinal muscular atrophy (SMA) protein, there remains much to be learned about specific mediators of this selective vulnerability. We have demonstrated that defects of the neuromuscular junctions (NMJs) are an early hallmark of SMA. The ability of motor neurons to form NMJs and thus control muscle activity may underlie the vulnerability of this neuronal population to reduced SMN in SMA. Moreover, our results indicate that motor neurons are especially sensitive to low SMN during early postnatal life, a period characterized by the development and refinement of the neuromuscular system. In contrast, depleting SMN at adult stages appears to have a relatively muted effect on muscles and nerves. In mice a brief window between PND12 and PND15 defines a critical period during which the neuromuscular system transitions from an SMN sensitive to resistant state. In this project we will use wild-type and novel inducible SMN knockdown mice to define precise molecular changes that occur during this period of development. We will also determine how reducing SMN selectively in the pre- and post-synapse affects the development of the mature neuromuscular junction. Collectively the experiments will determine 1) how a depletion of the SMN protein gives rise to the SMA phenotype and 2) serve as the basis of safe and effective treatments for the human disease.

Catarina M. Quinzii M.D.

RG Investigating the Pathogenesis of Encephaloneuromyopathy due to RMND1 mutations

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Mitochondria are often described as the “powerhouses of the cell” because these tiny structures generate most of the body’s energy by converting carbohydrates, fats, and proteins to water and carbon dioxide. Mitochondria are unique constituents of human cells because they are the products of two types of genetic

material: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Defects of either nDNA or mtDNA can cause mitochondrial dysfunction, which frequently affects brain and muscle, which require abundant energy. Disorders of mitochondrial protein synthesis have been reported in a heterogeneous group of patients, mostly presenting with early-onset, lethal diseases. Recently, in a patient with a new fatal, early-onset encephaloneuromyopathy and impaired mitochondrial protein translation, we identified a mutation in the gene encoding the required for nuclear meiotic division 1 (RMND1) protein, never before associated with a human disease. We will investigate why abnormal RMND1 causes mitochondrial dysfunction, by studying human RMND1 mutant and RMND1-depleted cells, murine RMND1-depleted embryonic stem cells, and a mouse model. Ultimately, we hope to develop a treatment for this devastating disease.

New York - Joan & Sanford I. Weill Medical College of Cornell University

Marilena D'Aurelio Ph.D.

RG Impaired amino acid metabolism in mitochondrial diseases: a target for therapy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Mutations in mitochondrial DNA (mtDNA) result in respiratory chain (RC) defects and energy metabolism impairment that affect multiple organs and manifest with severe neurological and muscular defects. Although the genetic defects are known, many aspects of the disease pathogenesis have yet to be elucidated. We identified changes in the levels of functionally relevant metabolites in cells with mtDNA mutations associated with severe mitochondrial encephalomyopathies. We found a defective metabolism of the amino acids glutamine and glutamate. Importantly, metabolic supplementation with specific amino acids improved the survival of the mutant cells. Our findings support the novel hypothesis that metabolic changes, due to forced glycolytic metabolism and to the blockage of pathways fueling substrates to the RC, are responsible for decreased glutamine uptake and glutamate availability in mutant cells. In this project, metabolic supplementation and genetic manipulations of key enzymes involved in the glutamine metabolism will be used to restore normal metabolite levels and improve the viability of mutant cells. The glutamine-glutamate metabolic pathway will be investigated in vivo in a mouse model of mitochondrial disease. Specific vulnerable tissues will be analyzed for amino acid metabolite levels. A metabolic supplementation diet will be used to bypass the defective enzymatic steps and improve the disease in the mouse model of mitochondrial myopathy.

Giovanni Manfredi M.D., Ph.D.

RG Store-operated Ca2+ entry and ER Ca2+ in mutant SOD1 astrocyte toxicity

$118,439.00	2/1/2014	1/31/2015	Year 2
$118,439.00	2/1/2015	1/31/2016	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a devastating neurological disorder that affects the neurons that control the muscles. The result of this disease is a fatally progressive paralysis. ALS is one of the most common forms of neuromuscular diseases and can be caused by genetic mutations (familial ALS) or occur sporadically. Recent developments have identified astrocytes, the cells that support motor neurons, as significant contributors to the disease in familial ALS caused by mutations in the superoxide dismutase 1 gene (SOD1), and also in the more frequent forms of sporadic ALS. Mutant astrocytes are likely to contribute to the death of motor neurons by secreting toxic substances. The mechanisms that cause these astrocytes to become toxic are unknown and will be the subject of this research proposal. We have developed a novel hypothesis to explain the mechanisms of astrocyte toxicity in familial ALS, which involves intracellular calcium signaling. Calcium is a fundamental ion that serves as an internal sensor for regulating many cellular functions. All cells, including astrocytes, have to keep calcium levels in check at all times. We propose that mutant astrocytes have impaired calcium regulation, leading to excessive secretion of substances, which in turn cause motor neuron toxicity. We will demonstrate this hypothesis and test approaches to normalize calcium regulation and astrocyte secretion to prevent motor neuron toxicity from astrocytes.

New York - Memorial Sloan-Kettering Cancer Center

Mary Baylies Ph.D.

RG Myonuclear Positioning: links to Nuclear structure and Muscle Function

$128,634.00	8/1/2014	7/31/2015	Year 3

Summary Emery Dreifuss Muscular Dystrophy (EDMD) has been linked to mutations in LMNA, a gene which encodes the Lamin A and C proteins. Lamin A and C are components of the nuclear lamina, a fibrous structure associated with the inner nuclear membrane via interactions with integral membrane proteins. Lamin A and C provide structural integrity and shape to the nucleus. They also interact with chromatin and transcriptional regulators to influence gene expression in myofibers and satellite cells. Recently, EDMD-linked mutations in Lamin A/C also have been shown to cause nuclear movement/positioning defects in tissue culture. Given the

many functions of Lamin A/C, the reason why LMNA mutations cause muscle disease remains unclear. We previously identified a microtubule-associated protein, Ensconsin (Ens) as critical for nuclear movement in both Drosophila and mouse muscle. ens mutant larvae do not move as fast as wild-type larvae, indicating that improper nuclear localization has significant impact on muscle function. We find that Ens physically and genetically interacts with Lamin C. Lamin C mutants have mispositioned nuclei and defective muscle function. We hypothesize that Ens and Lamin C act together, linking nuclear positioning to gene expression and muscle function. We will determine the nature of the interaction, how they regulate muscle function, and provide new insights to both the cellular processes required for optimal muscle function and to different muscle diseases.

New York - The Trustees of Columbia University in the City of New York

Eric A. Schon Ph.D.

RG Treatment strategies for human mitochondrial disease

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Mutations in mitochondrial DNA (mtDNA) are associated with classical mitochondrial disorders, as well as with Parkinson disease and normal aging. However, no general therapeutic strategies have been identified to combat diseases involving mtDNA mutations. We recently showed that in cells containing exclusively mutated mtDNAs that result in mitochondria with low membrane potential, it is possible to eliminate those mitochondria using rapamycin, a drug that activates autophagy (the cell's innate pathway for degrading unwanted materials, including "defective" mitochondria ["mitophagy"]). We have now found that in heteroplasmic cells (i.e. containing a mixture of normal and mutated mtDNAs, which is more typical of the clinical situation), rapamycin dramatically increases the proportion of "good" mitochondria and restores cellular bioenergetic function within only a few days, implying that induction of selective mitophagy of dysfunctional mitochondria could be a promising method to treat diseases involving a wide range of mtDNA mutations. We now propose to follow up on these exciting results, in two ways: (1) we will explore "functional shifting" using a broader range of informative compounds, and (2) we will try to understand the mechanism by which this effect occurs. Using these approaches, we hope to gain insight - both practical and basic - into novel approaches to treat mitochondrial myopathies.

Howard J. Worman M.D.

RG Emerin-LAP1 Interaction and X-linked Emery-Dreifuss Muscular Dystrophy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Mutations in two genes cause most cases of Emery-Dreifuss muscular dystrophy (EDMD). The X-linked form that affects only boys/men is caused by mutations in a gene known as EMD encoding a protein called emerin. Surprisingly, genetically engineered mice lacking emerin do not get muscular dystrophy or heart problems characteristic of EDMD, making preclinical research difficult. We have recently shown that emerin interacts with another protein called LAP1 and that the proteins act together in muscle. As LAP1 appears to compensate for loss of emerin in mice but not in humans, we have made new genetically engineered mice that lack both emerin and LAP1 from muscle. These mice get muscular dystrophy and heart disease that mimics what occurs in X-linked EDMD. We will use these mice to study abnormalities in muscle and test a potential new treatment for X-linked EDMD.

Rochester - University of Rochester

Robert Dirksen Ph.D.

RG Orai1 as a Therapeutic Target for Central Core Disease

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary There are no drug therapies available to treat central core disease (CCD) or environmental heat stress (EHS). The overall objective of this proposal is to evaluate the effectiveness of inhibiting calcium influx through store-operated calcium entry (SOCE) channels as a viable therapy to treat CCD and EHS. We will test the hypothesis that both the mitochondrial myopathy in CCD and excessive heat generation during EHS require uncontrolled calcium influx through SOCE channels. We will determine the effects of innovative mouse genetic and drug interventions to inhibit SOCE on the mitochondrial damage, core myopathy, and heat sensitivity of an established mouse model of EHS with central cores. Effects of SOCE inhibition on the heat stress response of normal mice will also be determined in order to assess the utility of SOCE channel inhibitors in preventing EHS and heat-related illness in normal individuals. In addition to CCD and EHS,

alterations in calcium homeostasis and mitochondrial function contribute to multiple other MDA-sponsored muscular dystrophies including Duchenne Muscular Dystrophy, Centronuclear Myopathy, Amyotrophic Lateral Sclerosis, Mitochondrial Myopathy, Myotubular Myopathy, Bethlem Myopathy, and Ullrich Congenital Muscular Dystrophy. Thus, the fundamental discoveries and therapeutic advances accomplished during this project will have broad implications for multiple MDA-supported muscle disorders.

Robert Griggs M.D.

HCTG FOR-DMD:Double-Blind Randomized Trial to Optimize Steroid Regimen in Duchenne MD

$64,260.00	10/1/2014	9/30/2015	Year 2
$85,680.00	10/1/2015	9/30/2016	Year 3

Summary This application requests funds for reimbursement for subject travel for an NIH-funded multicenter trial comparing long-term regimens of corticosteroids in boys with Duchenne muscular dystrophy (DMD). The corticosteroid prednisone is of established 18 months benefit to strength in DMD and another corticosteroid, deflazacort, may also be of benefit. Many corticosteroid regimens have been in use because of concerns regarding side effects and long-term risk/benefit, resulting in great variations in practice. This randomized controlled trial compares the 3 most widely used corticosteroid regimens to see whether both daily prednisone and daily deflazacort will be of greater benefit in terms of function and parent satisfaction than intermittent prednisone. The trial is randomizing 300 boys in North American and Europe aged 4-7 years to 0.75 mg/kg/d prednisone; 0.9 mg/kg/d deflazacort; or 0.75 mg/kg/d prednisone for 10 days alternating with 10 days off. Participants will be recruited over a 2 year period and followed for at least 3 years. The protocol includes standardized regimens for treatment and prevention of bone, cardiac, respiratory, behavioral, and cushingoid complications of DMD and corticosteroids. The average subject and his parent/guardian will have to stay overnight near the site to complete all procedures at each visit. It would be unfair to ask families to bear this cost. Therefore this application requests funds to reimburse North American families.

Charles Thornton MD

RG Models for therapeutic development in DM1

$94,393.00	8/1/2014	7/31/2015	Year 3

Summary The goal of this project is to expedite the development of effective treatments for myotonic dystrophy type 1. More specifically, we plan to use genetic engineering to develop mice that show the typical signs of myotonic dystrophy in skeletal muscle, so that new drugs can be tested for improvement of the muscular dystrophy in these animals.

Charles Thornton MD

CRNG Myotonic Dystrophy Clinical Research Network

$306,000.00	1/1/2015	12/31/2015	Year 3

Summary The goal of this project is to develop a Clinical Research Network that is focused on myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). Five centers will participate in the Network. The centers are distributed across the U.S. to maximize the opportunity for individuals with myotonic dystrophy to participate in research studies. Each center in the Network has a particular interest and expertise in clinical care and research on myotonic dystrophy. One of the main goals of the Network is to prepare for the testing of new treatments. The researchers in each center will work together to standardize the methods for evaluating the severity of myotonic dystrophy, and determine the best ways to assess whether new medications are having a beneficial effect.

NORTH CAROLINA

Chapel Hill - The University of North Carolina at Chapel Hill

Joan M. Taylor Ph.D.

RG Muscle development and repair mediated by the BAR-containing Rho GAP, GRAF

$132,000.00	2/1/2014	1/31/2015	Year 2
$132,000.00	2/15/2015	1/31/2016	Year 3

Summary We published that depletion of a skeletal muscle selective protein from developing tadpoles led to mobility defects and progressive muscle degeneration that was reminiscent of the disease progression observed in several congenital muscular dystrophies. We subsequently found that this protein acts to promote muscle formation and injury repair will identify the underlying mechanisms. Moreover, we found that this protein interacts with receptors frequently mutated in patients with muscular dystrophies, and will test the possibility that mis-regulation of this protein contributes to the debilitating nature of these diseases. We have developed several novel mouse models that will now enable us to test these exiting possibilities. These

studies will undoubtedly lead to new and important directions for therapies to target a multitude of congenital dystrophies.

Charlotte - Carolinas Healthcare Foundation

Amy D Harper MD

RRG Preparation for AAV9-mediated gene therapy for LGMD2I with FKRP mutations

$218,502.18	7/1/2014	6/30/2015	Year 1

Summary Mutations in FKRP gene are associated with a wide range of muscular dystrophies from mild limb-girdle muscular dystrophy (LGMD) 2I to severe Walker-Warburg syndrome (WWS) and muscle-eye-brain disease (MEB). Currently there is no effective treatment available. We have developed several mouse models with FKRP mutations representing the human disease. Adeno-associated virus (AAV) mediated gene therapy is one of the most promising and fundamental of the experimental therapies with the possibility to eventually cure the disease. In this proposal, we will test and determine the optimal promoter for muscle specific expression of the FKRP and for diminishing the expression of the transgene in liver for greater safety. We will also determine the long term efficacy of the viral sera type and the promoter system in the FKRP P448L mutant model, although this will take more than 1 year to complete. Our achievable goal is to obtain data for the efficacy and safety of the AAV9-FKRP in the mouse model for further advancing the therapy to clinic trials. In addition, we aim to determine clinical and biochemical outcome markers in patients with LGMD2I in preparation for a clinical trial with AAV9-FKRP gene therapy.

Charlotte - MDA Clinic & MDA/ALS Center at Carolinas Medical Center

CLAYTON S OWENS MSLS

SG 4th International Workshop for Glycosylation Defects in Muscular Dystrophies

$7,500.00	1/1/2015	4/30/2015	Year 1

Summary The focus of the 2015, 4th International Workshop for Glycosylation Defects in Muscular Dystrophy will be to address the potential for identifying drugs and therapies to upregulate functional glycosylation as well as AAV mediated gene therapy for FKRP-related diseases and the barriers being faced. As with the previous workshops, advances in clinical management and development of endpoint markers will provide a major topic of discussion. The workshop will bring together scientists and clinicians with active research in these areas to discuss the significance of the updated experimental data and facilitate potential collaboration to possibly identify new approaches to disease treatment. An area of special interest will be planning for future clinical research, including ways to quantify patient response to therapy and identify endpoints for research reporting. This invitational workshop will bring together an international group of approximately 25 highly respected clinicains and researchers for a igly interactive program. All participants will be presenting their research, and jointly they will seek ways to advance their own research as well as find areas for potential collaborative work.

Durham - Duke University

Charles Alan Gersbach Ph.D.

RG Genetic Correction of Duchenne Muscular Dystrophy with Engineered Nucleases

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Gene therapy is a promising approach to treating Duchenne Muscular Dystrophy (DMD). However, current methods typically require the integration of exogenous DNA into the genome or the lifelong re-administration of transient gene therapy vectors, both of which have significant safety and practical concerns. Furthermore, these strategies have been limited by an inability to deliver the large and complex dystrophin gene sequence. An exciting alternative to these gene replacement approaches is the targeted repair of the endogenous mutant dystrophin gene. This concept represents a potential cure to DMD without the need for permanent integration of or repeated exposure to foreign biological material. Engineered nucleases, including zinc finger nucleases and TALE nucleases, constitute powerful tools for coordinating the site-specific manipulation of genomic DNA sequences. The overall objective of this research proposal is to develop methods to repair endogenous mutant dystrophin gene sequences. The central hypothesis is that delivery of engineered nucleases to dystrophin-mutant cells will lead to gene restoration and reverse muscle degeneration. We are well prepared to undertake the proposed research because of expertise in designing and utilizing engineered nucleases and recombinases and in musculoskeletal gene therapy. Interdisciplinary collaborations with experts in gene-based therapeutics and translational medicine at Duke and UNC also strengthen this proposal.

OHIO

Cincinnati - Cincinnati Children's Hospital Medical Center - Research Foundation

Douglas Millay Ph.D.

DG Molecular control of mammalian myoblast fusion

$60,000.00	8/1/2014	7/31/2015	Year 2
$60,000.00	8/1/2015	7/31/2016	Year 3

Summary Fusion of myoblasts during skeletal muscle development and adult muscle regeneration is an essential step to form mutli-nucleated muscle fibers and functional muscle. Thus, myoblast fusion is an attractive candidate for therapeutic manipulation in muscular dystrophy although this approach has not been thoroughly tested. Additionally, understanding the mechanisms that govern fusion could benefit other therapeutic angles currently being investigated, such as cell-based therapies. The molecular mechanisms that allow mammalian myoblast fusion to occur at proper times and between appropriate cells remain unknown. We have identified a novel, muscle-specific gene that is an essential component for myoblast fusion. We will characterize the function of this factor in a mouse model of muscular dystrophy. Furthermore, we will unveil general mechanisms of mammalian myoblast fusion, expanding our knowledge of this process and identifying avenues for future therapeutic intervention.

Cincinnati - University of Cincinnati

Tom Thompson Ph.D.

RG Structural studies of myostatin inhibitors

$110,000.00	8/1/2014	7/31/2015	Year 3

Summary Treatments that improve muscle strength and mass are highly sought after to alleviate various forms of Muscular Dystrophy. Our bodies have a protein, myostatin, that naturally restricts the size of muscles. When myostatin is not functioning properly animals have greatly increased muscle mass. In addition, injection of inhibitors of myostatin cause massive increases in muscle. In fact, an antibody and separately a receptor decoy are being tested clinically for their effectiveness in increasing muscle mass and strength. Although these treatments have yet to be confirmed effective, they need to be injected and are difficult to produce, greatly increasing the cost of the treatment and the chance of harmful immune responses. Our bodies have proteins that naturally inhibit myostatin. The goal of my laboratory is to understand at the atomic level how these proteins neutralize myostatin.

Cleveland - Cleveland Clinic Foundation

Feng Lin Ph.D.

RG Development of a novel cell-based therapy for myasthenia gravis

$130,000.00	8/1/2014	7/31/2015	Year 3

Summary We recently developed a novel method to generate a special group of cells that markedly suppress immune reactions which lead to myasthenia gravis. Pilot studies indicate that this group of cells protect animals from experimental myasthenia gravis. We will try to understand how the migration and function of these cells are regulated, and to develop these cells as a new, effective treatment for myasthenia gravis.

Columbus - Research Institute at Nationwide Children's Hospital

Scott Q. Harper Ph.D.

RG Development of An Inducible FSHD Mouse Model

$108,748.00	2/1/2014	7/31/2015	Year 3

Summary Facioscapulohumeral muscular dystrophy (FSHD) is among the three most common muscular dystrophies. Although FSHD was formally classified in 1954, its cause is only now being defined. Specifically, several studies now support that FSHD is caused by expression of a gene called DUX4. The DUX4 gene is therefore a target for developing potential FSHD therapies. Animal models are major tools used to develop treatments for disease, but no FSHD-related animal models expressing DUX4 are currently published. This is a fundamental problem in the field. In this project, we will develop a mouse model that expresses human DUX4, as a potential model for FSHD. We hope this model could ultimately be used to develop treatments for the disease.

Columbus - The Ohio State University

Denis C Guttridge Ph.D.

SG 2015 Gordon Research Conference: “Myogenesis and Cellular Networks”

$5,000.00	4/1/2015	9/1/2015	Year 1

Summary The 2015 Gordon Research Conference on Myogenesis: Molecular and Cellular Networks will bring together 46 speakers that represent critical areas of skeletal muscle research with a total of 200 participants for a

five-day conference focusing on increasing our understanding of fundamental mechanisms of muscle development, regeneration, and homeostasis, which will inform on muscle related disorders such as muscular dystrophy and sarcopenia. The health relatedness of this application is that the discussions generated within this interdisciplinary conference will define current outstanding questions affecting human muscle health, by in-depth exploration of mechanisms regulating muscle development and muscle turnover, leading to new therapies based on these mechanisms.

Noah Weisleder Ph.D.

RG Protein therapy targeting limb girdle muscular dystrophy

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary Defective muscle cell membrane repair is associated with the progression of various types of limb girdle muscular dystrophy (LGMD) that is linked to mutations in many different genes in human patients. We recently discovered that Mitsuguimin 53 (MG53), a muscle-specific TRIM-family protein (TRIM72), is an essential component of the acute membrane repair machinery in striated muscle. MG53 acts to nucleate recruitment of intracellular vesicles to the injury site for membrane patch formation. We showed MG53 can interact with dysferlin to facilitate its membrane repair function. Results that are recently published establish that MG53 protein can be used directly as a therapeutic approach to increase membrane repair in skeletal muscle fibers. Our studies found that membrane injury leads to exposure of a signal to the extracellular space that can be detected by purified recombinant human MG53 protein (rhMG53). We generated in vivo data to show that intravenous delivery of rhMG53 can ameliorate cardiotoxin-induced damage to muscle fibers. Furthermore, we demonstrated that subcutaneous injection of rhMG53 could reduce the severity of pathology in the mdx mouse model of Duchenne muscular dystrophy. In this project we will test the capacity for the MG53 protein to reduce the pathology in animal models of three forms of LGMD. This application will represent a first resubmission of our application from December 2011 and contains additional preliminary data and a revised resarch plan.

Columbus - The Ohio State University Research Foundation

Stephen James Kolb M.D., Ph.D.

HCTG Motor Function Test Reliability in NeuroNEXT Infant SMA Biomarker Study

$69,492.00	6/1/2014	5/31/2015	Year 1
$56,009.00	6/1/2015	5/31/2016	Year 2
$57,851.00	6/1/2016	5/31/2017	Year 3

Summary The SMA Biomarkers Study in the Immediate Postnatal Period of Development Clinical Study (NCT01736553), enrolling through the NINDS NeuroNEXT Clinical Trial Network, seeks to define the natural history of motor function and putative SMA molecular and physiological biomarkers in infants during the first two years of life. This prospective study, which includes healthy infants, is designed to provide reliable, definitive baseline measurements that may be used in the design of future SMA interventional trials. Ensuring the reliability and validity of outcome measures is essential to the success of this study. The 15 enrolling study sites are expected to enroll at a low rate and visits are not frequent. Thus, it is important that retraining occur on a regular basis to maintain reliability and maintain technical standardization of motor test administration. Retraining will include education, remediation when needed, monitoring and reliability assessment. In this proposal, we request supplementary funding to allow for training of the motor function test evaluators for the duration of the study. We propose bi-annual retesting of all evaluators using videos produced specifically for this study. We also propose annual in-person meetings for all evaluators for retraining and inter-rater reliability testing of live infants.

Columbus - The Research Institute at Nationwide Children's Hospital

Jerry Mendell M.D.

CRNG MDA Clinical Network

$306,000.00	11/1/2014	10/30/2015	Year 3

Summary The overall goal for the proposed MDA DMD Clinical Research Network centered at Nationwide Children's Hospital (NCH) is to sustain and expand a network for the performance of critical natural history and pilot treatment trials in Duchenne muscular dystrophy. The clinical sites involved are led by experienced clinicians and clinical scientists with a demonstrated record of dystrophinopathy research. The network will provide a stable platform for the development and performance of trials including ongoing studies of cardiac natural history; pilot studies of spironolactone therapy; and treatment of infantile DMD with corticosteroids. No one center alone perform these studies, and the network proposed by the MDA represents an ideal approach to complete these stated goals.

Federica Montanaro Ph.D.

RG Defining the role of impaired Hedgehog signaling in DMD

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary In Duchenne muscular dystrophy (DMD), fibrosis and progressive failure of muscle regeneration are two major contributors to loss of motor function, progression of cardiac disease, and subsequent mortality. Therefore, intense research efforts are aimed at defining pathways that regulate fibrosis and regeneration in DMD, with the prospect of using this information to develop novel treatment approaches. Our laboratory has discovered for the first time that a signaling pathway called Hedgehog shows decreased activity in muscle biopsies from DMD patients, and in skeletal and cardiac muscle of mdx mice, a model of DMD. We further find that active Hedgehog signaling inhibits fibrosis while promoting muscle regeneration by activating muscle stem cells. Therefore, the goal of this study is to understand the consequences of decreased Hedgehog signaling for skeletal and cardiac muscle disease progression in DMD. In this project we will 1) study how decreased Hedgehog signaling affects muscle stem cells during muscle repair, and 2) test whether increasing Hedgehog signaling in the mdx mouse prevents loss of muscle tissue and preserves muscle function.

OREGON

Eugene - University of Oregon

Andrew Berglund Ph.D.

RG Stabilization of toxic RNA provides novel insights into myotonic dystrophy

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Myotonic dystropy is an RNA gain-of-function disease. When expressed, the toxic RNAs (CUG and CCUG repeats) sequester MBNL proteins and alter the levels and functions of other cellular proteins, causing an alteration of the splicing/gene expression and thus, disease. Specifically, the mis-splicing of MBNL targets are responsible for causing many of the symptoms associated with myotonic dystrophy. In this study we are using RNA modifications to stabilize the CUG/CCUG repeats in conformations that limit or eliminate the toxicity. Results from these studies will inform current therapeutic strategies and could lead to the development of novel therapeutic approaches for myotonic dystrophy.

Portland - Oregon Health & Science University

paul brehm Ph.D

RG Use-dependent fatigue in muscle rapsyn myasthenic syndrome is presynaptic

$117,216.00	8/1/2014	7/31/2015	Year 3

Summary We have identified zebrafish mutant lines that represent models for human neuromuscular diseases including a rapsyn-deficient myasthenic syndrome that forms the basis of this application. Rapsyn is a molecule that is responsible for localizing the acetylcholine receptor to the neuromuscular junction. Our zebrafish line twitch once provided the original identification of a rapsyn mutation as being causal to myasthenia and showed that muscle receptors were unable to localize to the synapse due to the mutation. It is widely assumed that muscle weakness in humans that results from mutant rapsyn is a direct consequence of the failure of receptors to localize. It certainly contributes to weakness but can't account for use-dependent fatigue, a hallmark feature common to many of the myasthenic syndromes. A potential solution was again offered by the twitch once zebrafish wherein nerve was defective and unable to reload and release transmitter in the normal time frame. This was completely unexpected because the mutant rapsyn is located in the muscle, not in the nerve. We now test a model whereby muscle synaptic activity is a key regulator of transmitter release by a retrograde signal from diseased muscle back to nerve. Because we have observed this phenomenon in other neuromuscular zebrafish mutant lines showing use-dependent fatigue, our findings call for a reassessment of the underlying mechanisms and treatment of those myasthenic syndromes.

PENNSYLVANIA

Philadelphia - The Children's Hospital of Philadelphia

Masahiro Iwamoto Ph.D.

RG Intervention of muscular dystrophy by selective RARgamma agonist

$135,000.00	2/1/2014	1/31/2015	Year 2
$135,000.00	2/1/2015	1/31/2016	Year 3

Summary It is well established that muscular dystrophy involves a progressive loss of muscle structure and organization and a progressive loss of muscle contractility, strength and function. It is also well established that the innate repair capacity of muscle is limited and is thus unable to counteract the inexorable progression and worsening of the disease over time. We are skeletal biologists who study the mechanisms of formation of normal and abnormal bone tissue and ways to treat common bone pathologies. In a recent series of studies, we made an unexpected and possibly breakthrough discovery. We were studying a disease called heterotopic ossification (HO) that involves formation of extra bone tissue at the expense of skeletal muscle. We found in animal models of HO that drugs called retinoid agonists were able to prevent formation of the extra bone tissue; at the same time, the drugs greatly stimulated the reparative capacity of adjacent muscle tissue. In this project, we will determine whether these drugs can in fact block or even reverse muscle degeneration in mouse models of muscular dystrophy, leading to a novel and powerful means of therapeutic intervention.

Philadelphia - The Trustees of the University of Pennsylvania

James Shorter Ph.D.

RG Generating Therapeutic Protein Disaggregases for Amyotrophic Lateral Sclerosis

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Here, we will generate therapeutic enzymes that reverse the clumping of specific proteins that is connected with ALS. If successful, our studies will provide a tool to reverse protein clumping in ALS and provide the foundations for new approaches to potentially treat ALS. The studies we propose will enhance our basic understanding of the importance of protein clumping in ALS, and whether targeting this process holds therapeutic potential. Our studies will ultimately increase our understanding of ALS for the ultimate benefit of patients. The therapeutic enzymes we propose to generate could have potential clinical applications. Our studies are essential to enhance our understanding of ALS and advance the development of potential therapeutics.

Hansell Stedman M.D.

RG Pattern Recognition Receptors in Muscular Dystrophy Pathogenesis and Therapy

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary This new project addresses a critical problem in the development of effective therapy for Duchenne Muscular Dystrophy and other causally related muscle diseases by integrating recent progress in several research fields. The problem is the immune response to gene transfer in the inflammatory environment of dystrophic muscle; the integrated fields of research include basic myology, immunobiology, surgical critical care, genetics, and virology. Recombinant gene transfer vectors based on the non-pathogenic adeno-associated viruses have shown great promise in murine models of muscular dystrophy. Attempts to translate this approach to canine disease models and humans have failed, while providing evidence for powerful immune responses to vector-associated antigens. Our new approach will harness emerging paradigms from the listed research fields to identify the earliest mechanisms activating this immune response in dystrophic muscle. This mechanistic information will identify rational targets for transient immunosuppression prior to vector administration, thereby improving the chances for safe and durable therapy for these devastating childhood onset diseases.

Lee Sweeney Ph.D.

RG Modulation of calcium handling in mouse models of muscular dystrophy

$92,750.00	8/1/2014	7/31/2015	Year 2
$93,983.00	8/1/2015	7/31/2016	Year 3

Summary A number of problems develop in the progression of muscular dystrophy that are potential targets to slow disease progression. It is becoming understood that in some muscular dystrophies, much of the muscle damage leading to muscle loss is due to improper “calcium handling” inside the muscle cells. This project will examine a peptide that has been shown to be safe in humans and has the potential to correct these calcium-handling defects, and thus slow the progression of a number of muscular dystrophies. We will test this peptide, CT38, in mouse models of DMD, Miyoshi myopathy, and myotonic dystrophy.

Pittsburgh - University of Pittsburgh

Stephen Meriney Ph.D.

RG A novel calcium channel agonist as a treatment for LEMS

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary The proposed experiments will evaluate the effectiveness of a novel drug we have developed as a potential treatment for Lambert-Eaton myasthenic syndrome (LEMS). This compound is a potent calcium channel agonist, and we will characterize the effects of this novel drug on calcium channel gating in cell lines expressing the types of calcium channels present at motor nerve terminals, on transmitter release and muscle force generation at LEMS model mouse neuromuscular junctions, and on a battery of behavioral tests of muscle strength using LEMS model mice. This new drug could be used in isolation, but since the properties of this new drug are hypothesized to work synergistically with the most commonly used treatment approach for LEMS (3-4 diaminopyridine, DAP), we propose that our new drug may be best used in combination with DAP, both enhancing DAP effects and allowing DAP to be used at lower doses that greatly reduce potential side-effects.

RHODE ISLAND

West Kingston - Gordon Research Conferences

Robert Dirksen Ph.D.

SG Muscle: Excitation/Contraction Coupling Gordon Research Conference

$7,500.00	5/31/2015	6/5/2015	Year 1

Summary This is a proposal for support of the only major international meeting dedicated to excitation/contraction (EC) coupling and how defects in this critical process underlie muscle disease. The two overarching objectives of the 2015 Muscle: EC coupling Gordon Research Conference are: Objective 1: To discuss and critically evaluate new breakthroughs regarding and unpublished studies on the pathophysiology of the different muscular dystrophies, as well as exciting new advances in target validation and the development of new therapeutics to treat congenital muscle disease. Objective 2: To promote visibility and leadership of junior investigators in the field by providing a platform for students, postdoctoral fellows, and new independent investigators to present their work, as well as to interact and network with established senior investigators. The 2015 GRC conference will consist of 35 speakers, 18 Discussion Leaders, and 16 poster presentations. Scientific sessions will focus on structural studies of the ECC apparatus, new insights into the molecular mechanisms and regulation of ECC, identification of new therapeutic targets, and the development of innovative treatments for muscle fatigue, sarcopenia, and muscle diseases that adversely impact millions of individuals in the US. For the first time for this GRC, the meeting will be preceded by a two-day Gordon Research Seminar (GRS) for graduate students and postdoctoral fellows.

TENNESSEE

Memphis - St. Jude Children's Research Hospital

Hong Joo Kim Ph.D.

DG Characterizing the role of pathogenic mutations of hnRNPs in IBMPFD and ALS

$60,000.00	2/1/2014	1/31/2015	Year 2
$60,000.00	2/1/2015	1/31/2016	Year 3

Summary Multisystem proteinopathy (MSP), formerly known as IBMPFD, is an inherited autosomal dominant disease characterized by broad phenotypic spectrum including Inclusion body myopathy (IBM), Paget's disease of bone (PDB), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). Valosin-containing protein (VCP/p97) is the only gene known to cause this pleiotropic disease so far. We have identified families with VCP-negative, autosomal dominantly inherited MSP. Using modern genomic technologies, we have succeeded in identifying pathogenic mutations in prion-like domains (PrLDs) of heterogeneous nuclear ribonucleoproteins (hnRNPA2B1 and hnRNPA1) as novel causes of this disease. hnRNPA2B1 and hnRNPA1 are RNA binding proteins involved in many aspects of mRNA metabolism and transport. The disease-causing mutations accelerate polymerization of purified protein and drive the formation of cytoplasmic inclusions in fly models that recapitulate the human pathology. We hypothesize that disease-causing mutations of hnRNPA2B1 and A1 lead to toxicity by loss of nuclear function and/or gain of toxic cytoplasmic function. To elucidate the role of hnRNPA2B1 and A1 on MSP pathogenesis, we are generating fly and mouse models expressing wild type or mutant form of hnRNPA2B1 and A1 protein. We seek to recapitulate the full spectrum of MSP, to elucidate the molecular mechanism of pathogenesis, and to identify targets genes that are misregulated in hnRNPA2B1 and A1 associated diseases.

TEXAS

Dallas - UT Southwestern Medical Center

Jeffrey Leigh Elliott M.D.

RRG SCO2 and SOD1 interactions in ALS

$22,260.73	9/1/2014	8/31/2015	Year 1

Summary In this project we plan to better understand the molecular basis for the mitochondrial toxicity for a certain class of ALS linked SOD1 mutants that are sensitive to oxidation and reduction. We believe that certain mutations in the SOD1 protein foster inappropriate interactions with another key protein called SCO2 that is critical for energy production in nerve cells. These findings would have great importance for those families with SOD1 mutations and familial ALS

Jeffrey Leigh Elliott M.D.

RRG SCO2 in SOD1 related ALS.

$40,000.00	4/1/2015	3/31/2016	Year 1

Summary Mutations in the gene encoding Cu,Zn superoxide dismutase (SOD1) account for one form of familial amyotrophic lateral sclerosis (ALS). This knowledge has enabled scientists to generate mouse models of the human disease which allow for study into the molecular pathways by which mutant SOD1 leads to motor nerve cell injury. Analysis of these mice suggests that a substantial part of mutant SOD1 toxicity may involve mitochondria or power house of the cell. My lab has extensively studied mutant SOD1 induced mitochondrial toxicity. We have found that mutant SOD1 interacts inappropriately with another protein, SCO2, which is a critical accessory protein in the normal function of cytochrome c oxidase (COX). COX is vitally important in the normal function of mitochondria, and with diminished or abnormal COX levels, specific mitochondrial dysfunction ensues. We will test the hypothesis that altering levels of SCO2 in live mice may impact motor nerve cell disease in mutant SOD1 transgenic models of ALS. Special mice with targeted loss of SCO2 levels or function can be bred with mutant SOD1 mice to obtain mutant SOD1 mice with lower SCO2 levels/function. Motor nerve pathology and disease and be assessed in these mice and compared to standard mutant SOD1 mice and other controls. Results will establish a critical pathway that goes awry in SOD1 related ALS, providing insights not only into mechanisms of disease but also into potential avenues for therapeutic intervention.

Houston - Baylor College of Medicine

Thomas A. Cooper M.D.

RG Therapeutic applications in cardiac and skeletal muscle mouse models of DM1

$100,000.00	8/1/2014	7/31/2015	Year 2
$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Pathogenesis of myotonic dystrophy (DM) occurs primarily through toxicity of the RNA expressed from the expanded allele; therefore the expanded repeat RNA is a critical therapeutic target. Mortality from DM type 1 (DM1) results primarily from disease manifestations in skeletal muscle (60%) and heart (25%). To develop mouse models for therapeutic and mechanistic studies in these two tissues, we generated transgenic mice that utilize a tetracycline (tet)-responsive transgene to inducibly express 960 CUG repeats in the context of a human DMPK genomic segment containing exons 11-15 (expressing the 3' terminal 1200 nt of the mRNA). Both models exhibit strong splicing changes and tissue abnormalities. The goals of this project are to develop these two models as well as a third model to express CUG repeat RNA in mouse cardiac conduction system, then perform systematic phenotypic characterization to determine endpoints for preclinical studies and use all three models for systemic delivery of modified antisense oligonucleotides (ASOs) not yet applied to DM1 models. Of particular interest in this project is to optimize delivery of ASOs to the heart, a substantial challenge for efficient ASO uptake. Upon completion of this project, we will have developed robust mouse models for DM1 heart and muscle and optimized delivery of therapeutic ASOs to these models.

Houston - Houston Methodist Research Institute (an affiliate of Weill Medical College of Cornell University)

Muralidhar L. Hegde Ph.D.

RG Novel role of TDP-43 in DNA strand break repair and implications to ALS

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary The genotoxicity of TDP-43, an hnRNP family protein primarily involved in RNA processing (but also binds to DNA) whose aggregation/toxicity have been etiologically implicated in Amyotrophic Lateral Sclerosis (ALS) is not investigated although the diseases associated with TDP-43 pathology accumulate significant genome

damage. Our preliminary data demonstrate that: (1) TDP-43 is required for efficient DNA double strand break repair (DSBR) in neuronal cells; (2) TDP-43 is recruited to DSB sites to stably interact with DSBR proteins, in neuronal cells; (3) TDP-43 depletion markedly increases DSB accumulation and sensitized human cells to DSB-inducing agents; (4) Nuclear-specific depletion of TDP-43 in neuronal cells cause increased DSB damage. (5) A strong correlation between TDP-43's nuclear clearance and the accumulation of DSBs in ALS-affected human postmortem spinal cord tissue. Based on these, we hypothesize that loss of nuclear TDP-43 in ALS, causes deficient repair of DNA strand breaks in neurons promoting cell death and thus deficient repair of DSBs is a key etiologic factor in ALS and other TDP-43 pathologies. These will be comprehensively tested in this project by pursuing three Specific Aims using state of the art biochemical, cell culture including primary neuronal culture) and mouse and human tissue. Achieving our goals will lead to a major paradigm shift in our understanding of ALS pathology will open up new avenues therapeutic interventions.

Houston - Methodist Neurological Institute

Stanley Appel MD

RG Immune Mechanisms in Amyotrophic Lateral Sclerosis.

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease with no significant therapeutic options. The clinical presentations are heterogeneous, as are disease onset and progression. Multiple genetic factors might explain this heterogeneity. Yet despite the diverse genes that initiate disease, neuroinflammation is a common denominator. Pathology is characterized by activated microglia and T cells that could mediate disease progression and contribute to disease heterogeneity. In mouse models disease follows defined pathways strongly influenced by the innate and adaptive immune systems; in early stages of disease injured motor neurons emit molecular signals initiating glial activation of anti-inflammatory M2 microglia and infiltration of regulatory T and Th2 cells to foster repair and neuroprotection. In later stages of disease, neuroprotection is transformed into cytotoxicity by proinflammatory M1 microglia and Th1 lymphocytes. These mouse data suggest that manipulating microglial and Treg levels and functions in ALS patients may potentially modify the outcome of the devastating neurodegeneration. The key questions to be addressed in this application are whether the phenotypes of monocytes and T lymphocytes in ALS patients parallel the changes noted in the SOD1 ALS mouse model, and whether these immune hallmarks reflect disease progression and could potentially be used as biomarkers for immunomodulatory therapies.

Houston - The University of Texas Health Science Center at Houston

Radbod Darabi MD., Ph.D

RG Optimization of Human ES/iPS based cell therapy for muscular dystrophies

$126,683.00	2/1/2014	1/31/2015	Year 2
$126,683.00	2/1/2015	1/31/2016	Year 3

Summary To date, there is no cure for Duchenne muscular dystrophy. Initial attempts to treat DMD with cellular therapies involved the transplantation of myoblasts, which was not successful. Because embryonic stem (ES) cells are capable of self renewal and differentiation capabilities, they represent an ideal cell source for therapeutic application. Especially, with the availability of adult cell reprogramming into ES like pluripotent stem cells (iPS cells) and the possibility of in vitro gene correction, there is a remarkable effort on using patient specific iPS based cell therapy for degenerative disorders including muscular dystrophies. Recently, by engineering human ES/iPS cells to express PAX7 (a master regulator of muscle adult stem cell- satellite cell- development), we have succeeded to generate human ES/iPS-derived myogenic progenitors endowed with great in vitro and in vivo regenerative potential. Here we plan to improve engraftment levels using strategies to improve cell delivery, and cell survival following transplantation. Also we aim to develop a non integrating viral or non viral transient gene delivery for PAX7 induction in human ES/iPS cells which moves our technology much closer for clinical applications.

Houston - The University of Texas Health Science Center at Houston

Mariah Rose Baker Ph.D.

DG Structural basis for EC coupling in the skeletal muscle CaV1.1 channel

$50,691.00	5/1/2014	4/30/2015	Year 1
$50,691.00	5/1/2015	4/30/2016	Year 2
$50,691.00	5/1/2016	4/30/2017	Year 3

Summary Ca2+ ions exert a profound influence on many physiological processes, including muscle contraction. Intracellular Ca2+ levels are regulated by a set of Ca2+ channels, specialized proteins that allow Ca2+ to cross cell membranes. Channel dysfunction leads to a wide array of muscle diseases and as such, they are targets for many drugs. Due to a high prevalence of muscle disorders, new ways to mitigate Ca2+ channel dysfunction are needed. Design of such strategies is limited by a lack of sufficient knowledge about the structure of these proteins. We study the skeletal muscle Ca2+ channel, CaV1.1, a voltage-gated integral membrane protein. Upon membrane depolarization CaV1.1 transmits a signal to the Ca2+ release channel, RyR1, which releases Ca2+ from intracellular stores to initiate muscle contraction. Structural information about these channels is essential to understanding molecular mechanisms underlying Ca2+ signaling. Our approach of electron cryo-microscopy will allow us to determine the 3D structure of a protein at <1nm resolution providing invaluable information about the molecular architecture and function of Ca2+ channels. We will utilize bioinformatics and protein modeling to enrich our structures. By combining techniques we aim to increase our knowledge of Ca2+ channel physiology, enhancing the understanding of the structure-function relationship of the CaV1.1 channel and laying the groundwork for novel strategies to ameliorate Ca2+ channel dysfunction.

UTAH

Salt Lake City - University of Utah

Nicholas Johnson M.D.

RG	A Longitudinal Study of Disease Progression in Congenital Myotonic Dystrophy			
	$125,000.00	8/1/2014	7/31/2015	Year 2
	$125,000.00	8/1/2015	7/31/2016	Year 3

Summary Congenital myotonic dystrophy presents with severe motor dysfunction and cognitive impairment in infancy. Currently, there is very little information about the range of symptoms, their rate of progression, functional disability, and quality of life in infants and children with congenital myotonic dystrophy. Recent data from preclinical models suggest antisense oligonucleotide (ASO) therapy may prove to be a very effective therapeutic approach for myotonic dystrophy type-1, and human clinical trials are anticipated shortly. If ASO therapy appears safe there will be great urgency to extend clinical trials to children with myotonic dystrophy, particularly those most severely impacted by the disease. In this project we will identify the most critical symptoms and how those symptoms change over time in congenital myotonic dystrophy to develop a model of symptom development and progression. This model will allow for appropriate symptoms to be targeted in future treatment trials, as well as determining the age of children who will benefit the most from future treatments. We propose to develop this model in children with congenital myotonic dystrophy from infancy to late childhood, evaluating their quality of life, cognition, speech, muscle strength, and gastrointestinal symptoms over a three year period.

WASHINGTON

Seattle - Fred Hutchinson Cancer Research Center

Zejing Wang M.D., Ph.D

RG	Gene therapy for treating cardiomyopathy in a dog model of DMD			
	$100,000.00	8/1/2014	7/31/2015	Year 2
	$100,000.00	8/1/2015	7/31/2016	Year 3

Summary Duchenne Muscular Dystrophy (DMD) is a fatal, X-linked muscular dystrophy affecting whole body skeletal and heart muscles in both humans and dogs. DMD is caused by the lack of functional dystrophin. There is currently no cure for DMD. Adeno-associated virus (AAV)-mediated delivery of micro version of the dystrophin (μdys) to skeletal muscle has shown promise in a DMD mouse model. However, few attempts have been made to treat DMD-associated cardiomyopathy, the leading cause of death in DMD. The only available treatments are medications for relieving symptoms of heart failure and heart transplantation. While treatment of skeletal muscle alone may improve disease in the treated compartments, the additional stress associated with subsequent increase in activity may accelerate heart injury and progression to heart failure. Hence, in this project, we will develop AAV-mediated gene therapy strategies in a preclinical DMD dog model that can then be applied to treat cardiomyopathy in human DMD patients. We will determine if transient immunosuppression, which we have shown to facilitate AAV delivery to skeletal muscle in dogs, enhances the efficiency of X-ray guided intracoronary AAV delivery to the heart. We will determine the therapeutic benefit of intracoronary delivery of AAV-mediated canine μdys to the heart in DMD. Efficient treatment of heart muscle will increase the likelihood of achieving the goal of effective gene therapy and the ultimate reduction of death in DMD patients.

Seattle - University of Washington

Joseph A Beavo Ph.D.

RG Proposal: Mechanism of sildenafil action in muscular dystrophy

$137,500.00	2/1/2014	1/31/2015	Year 2
$137,500.00	2/1/2015	1/31/2016	Year 3

Summary Recent results show that the classic PDE5 inhibitor, Viagra® (sildenafil), can ameliorate much of the cardiac pathology seen in the mdx mouse model of muscular dystrophy. Unfortunately, it is not clear how this drug works at a molecular level to improve cardiac function. In fact it is not even clear what the initial molecular target(s) are for Viagra®. Therefore, we propose to define the initial molecular target(s) for the PDE inhibitor drugs and to explore the molecular mechanisms by which Viagra® improves cardiac symptoms in this model. Answers to these questions are needed to properly interpret current and upcoming clinical trials and quite probably to better design follow-up clinical studies. Our recent studies, indicate that sildenafil both blocks the development of cardiac pathology and, more importantly, RAPIDLY reverses it after it has developed. More importantly, since the original application, we now know that Tadalafil, another PDE5 inhibitor, does NOT work. Therefore we feel that the most likely candidate is a direct effect on PDE1C in the cardiomyocyte itself. This is a novel mechanism not being addressed by other investigators in the field.

Jeffrey S Chamberlain Ph.D.

RRG Systemic Delivery of AAV vectors

$147,928.83	1/1/2015	12/31/2015	Year 1

Summary A number of approaches are currently being tested for therapy of Duchenne and Becker muscular dystrophies (DMD/BMD). Gene therapy represents an approach that would be applicable to all patients and is attractive due to its ability to fix the actual cause of the disorder: a defective dystrophin gene. Studies from our lab have shown that miniaturized dystrophin genes can be delivered to the muscles of animal models of DMD and that this delivery is effective at completely halting and, at a minimum, partially reversing pre-existing damage of diseased muscles. The most promising method to deliver “micro-dystrophin” genes involves delivery of AAV vectors into the bloodstream. Here we will test whether methods developed in mice can be applied to larger animals. If successful, the work would greatly simplify applying the methods in the clinic.

Joel R. Chamberlain Ph.D.

RG RNA interference-based treatment of FSHD modeled in mice

$110,260.00	8/1/2014	7/31/2015	Year 3

Summary Recent discoveries provide us with a clearer understanding of how the genetics of FSHD translate into disease. This new information defines a target for therapy development. We will both engineer a model and use existing models to test a novel therapeutic approach to eliminate disease pathology. Protein is made in FSHD muscle that results in muscle damage. The therapeutic approach we will take will reduce production of toxic protein to eliminate the FSHD muscle damage with a single application to muscles throughout the body. We have been working on this approach in other animal models of muscular dystrophy, and what we have learned will be applied to new FSHD mouse models for development of a potential treatment for FSHD.

Stephen D. Hauschka Ph.D.

RG New Regulatory Cassettes for Treating Diseased Muscle Tissues

$84,600.00	5/1/2014	4/30/2015	Year 1
$84,600.00	5/1/2015	4/30/2016	Year 2
$84,600.00	5/1/2016	4/30/2017	Year 3

Summary This project designs and tests on-off gene switches, so-called regulatory cassettes (RCs), for their use in treating neuromuscular disease. Most RCs function in all heart and skeletal muscles, but their low activity in cardiac, diaphragm, slow and intermediate fiber types needs improvement. This is particularly important in cardiac and breathing muscles, as these are often severely affected, and their poor function impacts longevity. After tests in muscle cultures, RCs are tested in mouse muscle disease models to determine if they express beneficial product levels. Our DMD studies entail collaborations with Guy Odom and Jeff Chamberlain to test their newest micro-dystrophins and micro–utrophins. These studies are critical due to intrinsic limits to the size of therapeutic genes that can fit into viral vectors. If an improved micro-dystrophin or micro-utrophin is too large to fit into a virus, our RCs require corresponding size reductions to accommodate the larger protein-coding region. Similarly, when smaller therapeutic proteins are designed, we design larger more active RCs so that fewer viruses are needed for patient therapy. This increases patient safety and lowers treatment costs. The best RCs are then checked for expression in human muscle cells and modified as necessary to retain high expression. An additional value of these studies is that our RCs can be used for expressing virtually any therapeutic protein or its micro-version for treating ANY neuromuscular disease.

Deok-Ho Kim Ph.D.

RG	Functional Restoration of Dystrophic Muscle using Bioengineered Cell Patches			
	$130,000.00	2/1/2014	1/31/2015	Year 2
	$130,000.00	2/1/2015	1/31/2016	Year 3

Summary This work aims to generate a functional muscle patch capable of providing long-term muscle strength and regenerative capacity, and improve morbidity in Duchenne Muscular Dystrophy (DMD) patients. The proposed nanopatterned muscle patch integrates novel approaches including nanotechnology, biolipid chemistry, stem and endothelial cell therapy. Muscle fibers will be engrafted on nanopatterned, biocompatible and controllably biodegradable materials conjugated with pro-survival biolipid that also promotes growth of blood vessels. This research is potentially applicable to treat DMD as well as other types of muscular dystrophies, or other debilitating muscles disorders.