

Active Research Grants

(ACTG)	Academic Clinical Training Grant
(CRTG)	Clinical Research Training Grant
(CRNG)	Clinical Research Network Grant
(DG)	Development Grant
(RG)	Research Grant
(SG)	Special Grant
(RRG)	Restricted Research Grant
(RIG)	Research Infrastructure Grant
(TR-IG)	Translational Research Infrastructure Grant
(MVP)	MDA Venture Philanthropy Grant

CALENDAR YEAR(s): 2013,2014,2015,2016

UNITED STATES ALABAMA

Birmingham - The University of Alabama at Birmingham Michael Miller Ph.D.

RG A unifying molecular mechanism for familial amyotrophic lateral sclerosis

\$116,711.00 2/1/2012 1/31/2013 Year 2 \$116,711.00 2/1/2013 1/31/2014 Year 3

Summary While only ten percent of ALS is inherited, investigating the genetic basis of inherited forms should lead to a better understanding of the sporadic form. A point mutation in the human gene vapb causes ALS or late-onset SMA, depending on the individual. We have been using worms and flies, which offer tremendous advantages for discovering gene functions, to identify the fundamental role of vapb relevant to motor neuron disease. Our results suggest that VAPB is a secreted factor that regulates mitochondrial functions important for motor neuron survival. We have evidence that ALS and SMA are caused by disruption of an evolutionarily ancient extracellular signaling mechanism that controls energy homeostasis in the adult motor neuron environment. The major impacts of this project are predicted to be the identification of 1) mitochondria as the disease linchpin and 2) drug targets from our suppressor screen. Our results in worms support the idea that mitochondrial defects can be reversed in adults. If true in humans, it may be possible to prevent or reverse disease progression.

Marek Napierala Ph.D.

RG	Correction of the Friedreich's ataxia gene defect using zinc finger nucleases.				
	\$111,188.00	2/1/2013	1/31/2014	Year 1	
	\$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 - The Translational Genomics Research Institute Lisa Baumbach Ph.D.

RG	Identification of New Disease Genes for Infantile Lower Motor Neuron Diseases				
	\$129,076.00	8/1/2012	7/31/2013	Year 2	
	\$129.076.00	8/1/2013	7/31/2014	Vear 3	

Summary Our research group has led a long-term effort to identify and collect families with Xlinked lethal infantile spinal muscular atrophy (XL-SMA; SMAX2: MIM 301830), a lethal infantile neurodegenerative disorder (similar to Type I SMA) with additional features of congenital contractures and fractures (also called "arthrogryposis"). Genetic mapping allowed identification of a candidate disease gene interval, which eventually led to identification of the first disease-associated mutations in a known gene, UBE-1 (Ramser et al, 2008), which catalyzes the initiating step in the protein ubiquitination pathway, which targets proteins for degradation. As an outcome of our current MDA grant, we have identified/collected 20 new cases of putative XL-SMA, and are completing UBE-1 mutation screening. Despite numerous phenotypic similarities of these patients with our previously reported UBE-1 mutation-positive XL-SMA cases, no new UBE-1 mutations have been detected. This raises strong suspicion of yet- to- be identified mutations and disease genes. Our long-term goal is to apply knowledge gained from XL-SMA disease gene discovery (and these investigations) to other related forms of infantile lower motor neuron disease (ILMD), thus improving prenatal and antenatal disease detection, as well as eventual therapeutic strategies. The goal of this application is to use several contemporary experimental approaches to identify novel disease genes for ILMD in this unique patient cohort.

Tucson - Arizona Board of Regents, University of Arizona Daniela Zarnescu Ph.D.

RG Gene and Drug Discovery in a Drosophila Model of ALS \$125,000.00 7/1/2012 6/30/2013 Year 3

Summary While the genetic causes of Amyotrophic Lateral Sclerosis (ALS) are just beginning to be discovered, the pathological features of this disorder have been extensively investigated and include motor neuron death, muscle atrophy and cellular inclusions that contain TDP-43 protein. With the recent identification of mutations in TDP-43, this protein has emerged as a common denominator for the majority of ALS cases known to date. TDP-43 is conserved in the fruitfly Drosophila and alterations in the expression of TDP-43 in neurons lead to neuroanatomical and locomotor defects similar to those found in human patients. Furthermore, the expression of mutant forms of TDP-43, which mimic those found in human patients, lead to neuronal loss and the formation of cellular inclusions. Here we propose to use this TDP-43 based Drosophila model for gene and drug discovery. Genetic screens will identify novel genes that interact with TDP-43, some of which may be involved in the etiology and/or the pathology of the disease. In addition, drug screens will identify pharmacological reagents that can rescue the neuroanatomical and locomotor defects in this Drosophila model. With this approach, we are well positioned to discover novel therapeutic targets and approaches for ALS.

Daniela Zarnescu Ph.D.

RG	Deciphering the role of insulin signaling in ALS				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	

\$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

Berkeley - The Regents of the University of California, Berkeley Jen-Chywan Wang Ph.D

RG	Mechanisms of Glucocorticoid-induced Muscle Atrophy				
	\$110,000.00	2/1/2012	1/31/2013	Year 2	
	\$.00	1/1/2013	12/31/2013	Year 3	

Duchenne Muscular Dystrophy (DMD) is an incurable genetic disease affecting one in Summary 3500 males in the United States. DMD is characterized by rapid progression of muscle degeneration, leading to loss in ambulation, paralysis, and death. Glucocorticoids are potent anti-inflammatory agents that are frequently used to treat DMD to delay and relieve these symptoms. While GCs are beneficial, chronic use of GCs can cause side effects, such as muscle wasting. Developing therapies that maintain anti-inflammatory activity of glucocorticoids with reduced muscle wasting will greatly benefit DMD patients. To do this, we first need to know how glucocorticoids cause muscle wasting. Glucocorticoids affect muscle biology by binding to a receptor protein, called glucocorticoid receptor (GR). The major action of GR is to alter the expression of a specific subset of genes. We have identified two GR-regulated genes that have been previously shown to affect muscle mass. In this proposal, we will study how glucocorticoids increase their expression. We also identified another eight GR-regulated genes that can affect a process controlling total protein amount in the cells. In this proposal, we will test whether increasing the expression of these genes can lead to muscle wasting and whether they are responsible for glucocorticoid-induced muscle wasting. Overall, these studies will help us to design improved glucocorticoid therapies with reduce muscle wasting effect for DMD patients.

Davis - The Regents of the University of California (University of California Davis) Samantha Harris Ph.D.

RG	Regulation of skeletal muscle contraction by myosin binding protein-C				
	\$122,012.00	8/1/2012	7/31/2013	Year 2	

Summary Distal arthrogryposes (DA) are a group of congenital disorders that cause muscle contracture (arthrogryposis) of distal limb segments, including the hands, fingers, and feet, leading to birth defects such as clubfoot, clenched fists, rotated joints, ocular disorders and other muscle abnormalities and weaknesses. The first genetic causes and disease genes linked to DA have recently been identified and include a number of muscle contractile and regulatory proteins, including myosin binding protein-C (MyBP-C). In heart, mutations in the cardiac version of MyBP-C are a leading cause of hypertrophic cardiomyopathy, the most common cause of sudden cardiac death in young people and a significant cause of heart failure in millions of people worldwide. However, despite intensive

investigation of cardiac MyBP-C, little is known regarding the related proteins, fast and slow skeletal MyBP-C, and how mutations in skeletal MyBP-C cause DA muscle contractures. The purpose of the proposed studies is to investigate the functional and mechanical properties of MyBP-C in skeletal muscle and to determine the effects of DA mutations on MyBP-C function and its ability to regulate skeletal muscle contraction.

David Paul Richman M.D.

RG	Pathogenesis of Anti-MuSK Myasthenia					
	\$137,500.00	2/1/2013	1/31/2014	Year 1		
	\$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.

Irvine - The Regents of the University of California (Irvine) Virginia Kimonis M.D., MRCP

RG	Preclinical Studies in the VCP knock-in mouse model of hIBM				
	\$124,000.00	7/1/2012	6/30/2013	Year 3	

Summary hIBM is characterized by progressive muscle weakness and atrophy of the skeletal muscles beginning usually in the 40s. Although the primary gene defects underlying the disease have been reported to be missense mutations in the Valosin Containing Protein (VCP) gene, its molecular and tissue pathogenesis are still to be clarified. Therefore, we aim to characterize the development of molecular and tissue pathogenesis as well as muscle weakness using new mouse models made with the knock-in mouse model carrying the most common VCP-disease mutation (R155H). We will perform studies of the ER (endoplasmic reticulum) associated degradation pathway in order to identify the cause and treatment of the vacuoles, a prominent aspect of this disorder. Additionally, we will clarify the effect of physical exercise to inhibit the progression of muscle weakness in mutant mice. These analyses are important steps in order to understand the pathogenesis of IBM which in turn is an important step towards understanding the basis of the disease in other muscle diseases.

Tahseen Mozaffar M.D.

SG	Third Annual UC Irvine Neuromuscular Colloquium				
	\$5,000.00	5/17/2013	5/20/2013	Year 1	

Summary The Third Annual UC Irvine Neuromuscular Colloquium is a national academic meeting of neuromuscular physicians, scientists and clinician-scientists. This conference has been a highly successful gathering of regional neuromuscular experts and allows an opportunity for these experts to network, discuss recent advances in the field, discuss challenging and interesting neuromuscular patients and develop research collaborations and ideas. This is a one-day conference, with didactic lectures and case presentations, as well as short research talks. Preliminary agenda attached.

La Jolla - Amicus Therapeutics, Inc. - La Jolla Eric Sjoberg Ph.D

RG Can Pharmacological Chaperones Inhibit rhGAA ERT Induced Immunogenicity

\$111,256.00	2/1/2012	2/1/2013	Year 1
\$75,116.00	2/1/2013	2/1/2014	Year 2

Enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase Summary (GAA) is the only approved therapy for Pompe disease. The majority of Pompe patients on ERT develop antibodies against GAA that can severely limit tolerability and efficacy. While patients that produce no enzyme develop high titer antibody responses more readily than patients that express low levels of enzyme, there are reports of adult-onset patients (that express functional enzyme) with high antibody titers. This suggests that specific HLA types within the patient population may be driving a portion of this immune response. Denatured proteins are more immunogenic than their properly folded counterparts. GAA rapidly denatures in the neutral pH environment of the plasma, the site in which the ERT is administered, which may lead to the ERT-mediated immunogenicity. Pharmacological chaperones are small molecules that selectively bind and stabilize the target enzyme. We have developed a specific pharmacological chaperone for GAA, AT2220 that stabilizes the enzyme at neutral pH. By stabilizing GAA in plasma, AT2220 may mitigate the immune response associated with ERT treatment. In this work, we will use an ex vivo assay of normal and Pompe patient human PBMCs challenged with ERT and/or AT2220 to determine: 1) if a link exists between HLA types and ERT-mediated immunogenicity, and 2) if AT2220 can mitigate ERT-mediated immunogenicity.

La Jolla - Ludwig Institute for Cancer Research Ltd Don Cleveland Ph.D.

RG	Determining the contribution of mitochondrial dysfunction in ALS pathogenesis			
	\$153,699.00	8/1/2012	7/31/2013	Year 2
	\$133,981.00	8/1/2013	7/31/2014	Year 3

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative Summary disorder, characterized by the selective loss of motor neurons and skeletal muscle wasting. Although the mechanism underlying the premature degeneration and death of neurons during ALS is still unknown, evidence from many experimental directions has supported the proposal that a central feature of ALS is damage to mitochondria, the intracellular organelles that consume oxygen to produce the chemical fuel that powers all cell functions. Indeed, abnormal mitochondrial morphology, deficits in the ability of mitochondria to produce chemical energy, and damage to those mitochondria so that they produce toxic forms of oxygen have consistently been reported in ALS patients and mouse models that are genetic mimics of an inherited form of ALS. To determine the cell types of the central nervous system in which mitochondrial damage occurs and whether increasing mitochondrial activity alters ALS pathogenesis, genetic methods in ALS model mice will be used to isolate specific cell types of the nervous system to assess damage to the mitochondria and to determine whether increasing mitochondrial activity can delay ALS disease course. This approach should resolve the nature and cellular localization of mitochondrial damage in ALS pathogenesis and whether rescuing this damage can alter disease, thus providing potential directions for therapies.

Adrian Israelson Ph.D.

DG	Identifying the mechanism of mutant SOD1 misfolding and toxicity in ALS				
	\$60,000.00	2/1/2012	1/31/2013	Year 2	
	\$60,000.00	2/1/2013	1/31/2014	Year 3	

Summary Accumulated evidence suggests that crucial features of ALS are protein misfolding leading to protein aggregation and mitochondrial damage and dysfunction. Indeed, these features have been previously reported in both ALS patients and animal models that are genetic mimics of an inherited form of ALS. Of the familial cases, about 20 % of them are attributed to a toxic property of a mutation in superoxide dismutase 1 (SOD1). It has been 17 years since the finding that mutant SOD1 causes ALS; however, the specific mechanism for toxicity to motor neurons remains unknown. Several groups have shown that a proportion of these mutants are misfolded and are stably bound to the cytoplasmic face of spinal cord mitochondria, but how this association occurs remains unclear. I aim to determine the

mechanism by which these misfolded mutants aggregate and damage mitochondria selectively in specific affected tissues.

Clotilde Lagier-Tourenne M.D., Ph.D.

DG	Determining RNA metabolism alterations in Amyotrophic Lateral Sclerosis				
	\$60,000.00	8/1/2012	7/31/2013	Year 2	
	\$60,000.00	8/1/2013	7/31/2014	Year 3	

Summary Recent identification of ALS-causing mutations in two genes encoding for TDP-43 and FUS/TLS, respectively, has initiated a paradigm shift in understanding ALS pathogenesis. Both TDP-43 and FUS/TLS are RNA binding proteins, suggesting alterations in RNA processing as key in ALS. It is now fundamental to decipher the precise roles of TDP-43 and FUS/TLS in RNA metabolism regulation and how alterations in them may underlie ALS pathogenesis. I will use state of the art methods in sequencing to obtain an unbiased map of TDP-43 and FUS/TLS RNA targets and I will determine RNA modifications in novel existing mouse models and in motor neurons derived from iPS cells of ALS patients. This approach will identify candidate genes whose altered processing is linked to neurodegeneration. Importantly, TDP-43, which is mutated in a restricted number of ALS patients, is also abnormally aggregated in most of ALS sporadic cases and in other neurodegenerative disorders. The central hypothesis underlying this work is that understanding of TDP-43 and FUS/TLS normal functions and pathogenic properties will serve as the foundation for development of potential therapeutic approaches for the vast majority of ALS patients.

La Jolla - Sanford-Burnham Medical Research Institute Pier Lorenzo Puri M.D. Ph. D.

RG	Signal-dependent control of gene expression in satellite cells				
	\$103,112.00	2/1/2012	1/31/2013	Year 2	
	\$103.112.00	2/1/2013	1/31/2014	Year 3	

Summary The elucidation of the mechanism that controls gene expression in satellite cells (SCs) exposed to signals emanated by the regeneration environment is an important gap of information to fill in order to understand the molecular basis of the Duchenne Muscular Dystrophy (DMD) progression, in relationship with the decline of the regenerative response of dystrophic muscles at advanced stages of disease. It is also the prelude for interventions toward stimulating therapeutic regeneration of dystrophic muscles by endogenous stem cells. And it provides key information to devise strategies for SC expansion and cell mediated-transplantation in the treatment of DMD. We have identified a novel pathway linking the inflammatory component of the SC niche with the "epigenetic" network that regulates SC proliferation and differentiation. This pathway is triggered by one inflammatory cytokine highly expressed in dystrophic muscles - TNF alpha - and is converted by the p38 signaling to the Polycomb repressive complex 2 (PRC2) into epigenetic signals that control the expression of Pax7 – a key regulator of SC identity proliferation and differentiation. We will investigate the integrity of this signaling in SC from dystrophic (mdx) mice at different stages of diseases and will identify new targets for interventions toward manipulating this signaling to promote regeneration of dystrophic muscles.

Alessandra Sacco Ph.D.

RG	In utero and neonatal stem cell therapy for Duchenne Muscular Dystrophy				
	\$148,777.00	8/1/2012	7/31/2013	Year 2	
	\$148.777.00	8/1/2013	7/31/2014	Year 3	

Summary Duchenne muscular dystrophy (DMD) starts inevitably at birth, and by the time patients are diagnosed and begin their treatment, irreversible damage has already accumulated in skeletal muscle and its function is permanently lost. This aspect poses a major hurdle to cell-based therapies in young adults, as they face the challenge of rescuing permanently damaged tissue. We hypothesize that therapeutic intervention during fetal and neonatal stages will overcome this roadblock, as no major injury has been inflicted to the tissue, dissemination of cells throughout developing muscles will be more efficient, and immune tolerance towards donor cells can be induced. Accordingly, in this project we will develop in utero and neonatal muscle stem cell (MuSC) transplantation strategies in dystrophic

animal models and evaluate cell survival, proliferation and migration of donor cells within host muscles, induction of immune tolerance and finally assess the therapeutic improvement in muscle function. To this aim, we will employ three powerful tools we recently developed: (1) An approach to prospectively isolate adult MuSC, (2) A noninvasive bioluminescence imaging assay to monitor the dynamic behavior of MuSC in vivo, and (3) A novel mouse model of DMD, mdx mice lacking telomerase activity, that closely mimics the clinical progression in humans, ideally suited for testing potential therapies.

La Jolla - The Regents of the University of California, San Diego Ju Chen Ph.D.

RG The Role of Cypher in skeletal muscle function and disease

\$110,000.00 7/1/2012 6/30/2013 Year 3

Summary Mutations in Cypher result in myofibrillar myopathy (MFM) and late-onset distal myopathy. Studies of patients with myotonic dystrophy (DM), the most common adult form of muscle dystrophy, have demonstrated impaired splicing of Cypher isoforms in skeletal muscle tissues. Cypher is also significantly down-regulated in mice exhibiting skeletal muscle atrophy. These observations suggest that Cypher plays essential roles in skeletal muscle function and disease. A better understanding of the in vivo function of Cypher and its isoforms is key to developing potential therapies for Cypher-based MFM and potentially other myopathies. In this project, we will conduct a series of studies which will help us to understand biological functions of Cypher and its isoforms at molecular, cellular, and physiological levels and thus gain insight into mechanisms by which mutations in Cypher cause myopathies, thereby improving our general understanding of myopathy. In addition, the mouse lines will be useful as test models for potential therapies.

Adam Jeffrey Engler Ph.D.

RG	Mechanically programmed adipose-derived stem cells to treat muscular dystrophy				
	\$130,000.00	8/1/2012	7/31/2013	Year 1	
	\$130,000.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Masahiko Hoshijima M.D., Ph.D.

RG	Genetic treatment of cardio-respiratory failure in muscular dystrophy				
	\$122,462.00	2/1/2012	1/31/2013	Year 2	
	\$122.462.00	2/1/2013	1/31/2014	Year 3	

Summary In patients with muscular dystrophies including the Duchenne/Becker Muscular Dystrophies and Limb-Girdle Muscular Dystrophies (LGMDs), both skeletal and cardiac muscles are severely affected. While progressive weakness of neck, trunk and limb muscles disables these patients, their major causes of death are cardiac and respiratory failure. Using adeno-associated viral vector-based gene therapy, we recently became successful to treat cardiac and respiratory failures of BIO14.6

hamsters, an animal model of muscular dystrophy and inherited cardiomyopathy, at their advanced disease stage and substantially elongate their lifespan. Notably, muscle defects in BIO14.6 hamster is caused by the genetic defect of the delta-sarcoglycan, a membrane protein, mutations of which have been linked to a sub-type of human LGMD. Nonetheless, previous studies including ours have not determined how therapies affect respiratory and cardiac failures interactively. The current project takes advantage of recent advancement in cell-type specific gene transfer technologies and investigates (1) how cardiac specific genetic correction affects respiratory function and (2) whether skeletal muscle selective gene replacement therapy alters heart function. The project will provide new knowledge that guides us to understand how pernicious cardiac and respiratory dysfunctions in muscular dystrophy should be collectively treated.

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

RG Modeling motor neuron degeneration in SBMA \$110,000.00 7/1/2012 6/30/2013 Year 3

Spinal and bulbar muscular atrophy (SBMA, Kennedy's disease) is an adult onset neuromuscular disorder affecting only men. We wish to understand why motor neurons are dying in this disease. Toward this end, we have created a highly representative mouse model of SBMA and have produced neuron cell culture models of SBMA. We have used these models to understand why motor neurons degenerate in SBMA. Indeed, our studies thus far have yielded important leads as to candidate pathways that are crucial for motor neuron degeneration. We wish to build on our previous findings to better understand the mechanistic basis of the motor neuron disease in SBMA, as the pathways that we define will be crucial targets for therapy development, not only in SBMA but also in all related motor neuron diseases.

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

RG	SBMA motor neuron degeneration: molecular basis and therapy				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Alysson Renato Muotri Ph.D.

RG	Modeling Familial ALS8 with Human Induced Pluripotent Stem Cells				
	\$120,822.00	2/1/2012	1/31/2013	Year 2	
	\$120,822.00	2/1/2013	1/31/2014	Year 3	

Summary ALS is a complex disorder, involving multiple cellular and genetic targets. At present, most hypotheses describing the molecular and cellular basis of ALS are based upon mouse models, studies of post-mortem pathology, correlative neurological data, imaging and culture of immortalized lymphoblasts or fibroblasts. The inability to isolate populations of motor neurons from living subjects has hindered the progress toward studying the underlying mechanisms of many neurologic diseases.

Studies of cadaver tissue are often of limited use, especially for neurodegenerative disorders where the onset of disease usually precedes death by years, and show only the end stage of the disease. In addition, frozen tissue sections are of limited use for studying cellular physiology and neural networks. Animal models often do not recapitulate all aspects of complex human diseases. The iPSC technology provides a promising approach to this problem as they allow the genomes of human subjects afflicted with ALS to be captured in a pluripotent stem cell line. Such cells can then be differentiated to human motor neurons or glial cells to evaluate whether the captured genome with specific gene defects, alters neuronal or glial phenotype. An iPSC model may also address human specific effects avoiding overexpression systems and some aspects of the well-known limitations of animal models, such as the absence of a human genetic background.

G. Diane Shelton D.V.M, Ph.D.

SG 18th International Congress of the World Muscle Society \$10,000.00 10/1/2013 10/5/2013 Year 1

Summary The Specific Aims of the annual Congress of the World Muscle Society are to bring together established and new researchers, and clinicians working in the neuromuscular field for stateof-the-art reports and discussions on timely selected topics related to muscle disease, and to learn of new developments across the neuromuscular field including potential new therapeutics. This is an international Congress and the only annual meeting on this topic that brings together investigators and clinicians from around the world. WMS 2013 is of particular importance for young investigators in the USA, as economic considerations may preclude their attendance at overseas international meetings. Neuromuscular disease is a rapidly expanding field and the annual meetings of the WMS promote dissemination of important new information relevant to the goals of the MDA. The Asilomar venue should be ideal in promoting discussions between new and established investigators through the extensive poster sessions which are a large portion of this meeting and through attendees all residing in the same location. The last meeting of the WMS in the USA was in 2001 in Snowbird Utah, so the 2013 meeting is a wonderful chance for investigators in the USA to showcase interest and activity in this field. Support for this meeting through the MDA would further enhance the importance of this meeting to international attendees.

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	2/1/2013	1/31/2014	Year 1	
	\$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	n, and Function of Gamr	na-Actin in the Sarcopla	plasmic Reticulum		
	\$60,000.00	8/1/2012	7/31/2013	Year 1		
	\$60,000.00	8/1/2013	7/31/2014	Year 2		
	\$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	2/1/2013	1/31/2014	Year 1	
	\$60,000.00	2/1/2014	1/31/2015	Year 2	
	\$60.000.00	2/1/2015	1/31/2016	Year 3	

Our aim is to identify molecules that could be developed into therapeutics for Summary 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				
	\$59,271.00	8/1/2013	7/31/2014	Year 1	
	\$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	2/1/2013	1/31/2014	Year 1
\$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	Dystrophin Gene Editing Strategies for Duchenne Muscular Dystrophy					
	\$136 305 00	7/1/2012	6/30/2013	Vear 3		

Summary Our research group has pioneered a technology that seeks the use of small molecules called oligonucleotides to act directly on the source of the problem the DNA. DNA contains the information needed by every cell, including muscles, to function properly. In Duchenne muscular dystrophy patients the DNA that makes up the dystrophin gene contains errors. We 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 increase the efficiency of the repair to levels suitable to treat the disorder. At first we intend to test a number of different oligonucleotide structures in order to determine the most effective. Secondly we will test these structures for their efficiency to correct different dystrophin mutations in animal models for DMD. Each one of these steps is necessary to ensure a safe and effective treatment to human patients.

Carmen Bertoni Ph.D.

MVP	Preclinical Investigation of RTC#13 for the Treatment of DMD			
	\$76.257.00	8/1/2012	9/30/2013	Year 3

Summary Duchenne muscular dystrophy (DMD) is a genetic disease caused by mutations in the dystrophin gene that leads to absence of dystrophin expression in muscles. Many of the mutations that cause DMD are so-called nonsense mutations. They are generally caused by single point mutations in the dystrophin gene that lead to the inappropriate presence of specific sequences (UAA, UAG or UGA) called stop codons. These stop codons cause a premature arrest in the synthesis of the dystrophin protein. As a result, no dystrophin is produced in skeletal muscles and heart. RTC#13 is an experimental drug that has been identified by the laboratory of Dr. Richard Gatti at the University of California Los Angeles (UCLA). We have recently shown that this drug can restore dystrophin expression in muscles of mdx, a mouse model for DMD. Our goal is to determine whether this compound is safe to use in patients and to optimize the dose necessary to achieve therapeutic effects in DMD boys.

Carmen Bertoni Ph.D.

RG	Gene Editing of Dystrophin for the Treatment of Duchenne Muscular Dystrophy				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Giovanni Coppola MD

RG	Peripheral Biomarkers in Friedreich's Ataxia				
	\$132,847.00	2/1/2012	1/31/2013	Year 2	
	\$132.847.00	2/1/2013	1/31/2014	Year 3	

Summary The availability at the preclinical stage of multiple compounds with therapeutic potential in Friedreich's ataxia increases the need for better and more sensitive markers of disease severity and progression. We and others have used gene expression data from peripheral blood in order to gain insights into the pathogenesis of diseases of the central nervous system, such as Friedreich's ataxia. In a pilot study, we identified a biomarker set of 77 genes which show changes correlated with disease status in 10 patients, 10 controls and 10 carriers. We propose to validate our preliminary data by studying a 10-times larger cohort over three years, in collaboration with 2 of the largest Ataxia Centers in the country: UCLA and the Children's Hospital of Philadelphia. The rarity of diseases like Friedreich's ataxia constitutes a challenge for large-scale biomarker and natural history studies. We propose to collect and store DNA and RNA from these subjects and to make the gene expression data available in a web-based database, providing the scientific community with the first centralized repository of gene expression data in Friedreich's ataxia.

Rachelle H. Crosbie-Watson Ph.D.

RG	Evaluation of sarcospan treatment in muscular dystrophy				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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	8/1/2013	7/31/2014	Year 1		
	\$100,000.00	8/1/2014	7/31/2015	Year 2		
	\$100,000.00	8/1/2015	7/31/2016	Year 3		

Our ability to breathe, move, and interact with the world depends on the function of Summary 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	Investigation of osteopontin and inflammatory processes in mdx mice					
	\$125,000.00	8/1/2012 7/31/2013	Year 2			
	\$125,000.00	8/1/2013 7/31/2014	Year 3			

Summary Immune cells enter damaged muscle in order to help clean up debris and facilitate muscle repair. In the case of DMD and the mdx mouse, immune cells invade and then persist in the muscle, due to the chronic, damaged state that exists. The continuous presence of immune cells leads to increased scar tissue formation, due to the chemicals secreted by immune cells. Our studies have identified a protein called osteopontin that directs the immune cells to enter dystrophic muscle. By targetting this protein in mice, we have shown that both inflammation and scar tissue formation are reduced and the disease is greatly improved. The studies proposed in this investigation are designed to gain insight into specific ways in which osteopontin affects the immune cells that enter mdx muscle, and mechanisms involved.

Melissa Spencer Ph.D.

RG	Mechanisms involved in calpainapathies				
	\$130,000.00	8/1/2012	7/31/2013	Year 1	
	\$130,000.00	8/1/2013	7/31/2014	Year 2	
	\$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	8/1/2013	7/31/2014	Year 1
	\$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 "nontriggered" 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.

Oakland - Children's Hospital & Research Center Oakland Julie Saba M.D.

RG	Sphingosine-1-phosphate signaling in muscle regeneration and homeostasis				
	\$132,005.00	2/1/2012	1/31/2013	Year 1	
	\$130,231.00	2/1/2013	1/31/2014	Year 2	
	\$130,231.00	2/1/2014	1/31/2015	Year 3	

Summary Enhancing muscle regeneration and muscle stem cell functions may provide a new strategy for treating muscular dystrophy (MD). Sphingosine-1-phosphate (S1P) is a lipid that stimulates cell signals that promote muscle cell survival and activate muscle stem cells. Our previous genetic studies established that S1P metabolism is important in maintaining normal muscle development and homeostasis. Importantly, when we decrease S1P levels in mice using drugs or genetic approaches, we see a corresponding decrease in muscle stem cell activation and muscle regeneration after injury. Our laboratory has also observed that S1P signaling and metabolism are activated during muscle injury but may be deficient in muscles affected by MD, thereby contributing to poor muscle regeneration. In contrast, when we use a food-derived small molecule that causes accumulation of S1P, we observe improved muscle regeneration and stem cell functions in a mouse model of MD. Our findings suggest that stimulating S1P signaling may improve muscle regeneration and strength in patients with MD. In our project, we will: 1) study effects of modulating S1P signaling on muscle stem cell growth, activation and gene expression using S1P inhibitors, activators and genetic approaches, 2) measure S1P levels in muscles and blood of control mice and MD mouse models during disease progression, and 3) test the effect of modulating S1P levels on the pathological and clinical indicators of disease progression in MD mouse models.

Palo Alto - Palo Alto Institute for Research & Education, Inc. Thomas Rando M.D., Ph.D.

RG	Mechanisms of Fibrosis in Muscular Dystrophies				
	\$125,000.00	8/1/2012	7/1/2013	Year 2	
	\$125,000,00	8/1/2013	7/31/2014	Year 3	

Summary Fibrosis refers to the development of scar-like tissue in place of functional cells of that tissue. In skeletal muscle, fibrosis develops as muscles waste from degenerative disorders, such as muscular dystrophies, and muscle cells are replaced by connective tissue. This is associated not only with progressive weakness as functional muscle cells are lost, but also by progressive muscle stiffness since connective tissue is not as elastic as muscle tissue. The goals of the experiments described in this proposal are to understand why fibrosis occurs in the muscular dystrophies and to determine the biochemical mechanisms that lead to that fibrosis. We have preliminary data that suggests that a specific biochemical pathway, known as the "TGF-beta signaling pathway", is activated in dystrophic muscle and affects muscle stem cells in a way that leads to the development of fibrosis. We will directly test whether blocking this pathway leads to a reduction of the fibrosis that develops in the mdx mouse. These studies have the potential to lead directly to new therapies that will reduce the amount of fibrosis in the muscles of boys with Duchenne muscular dystrophy.

Palo Alto - Stanford University

Andrew Tri Van Ho Ph.D.

DG	Treatment of mdx/mTR Model of DMD with Human Muscle Stem Cells					
	\$60,000.00	2/1/2012	1/31/2013	Year 1		
	\$60,000.00	2/1/2013	1/31/2014	Year 2		
	\$60,000.00	2/1/2014	1/31/2015	Year 3		

Summary Current therapeutic approaches for duchenne muscular dystrophy (DMD) merely treat the symptoms of the disease. However, recent progress in mice suggests that transplantation of mouse muscle stem cells could ameliorate the debilitating effects of skeletal muscle wasting. In this project, we will develop isolation procedures and characterize human muscle stem cells from human muscle biopsies. Using a bioengineering approach, we will systematically study the influence of candidate biomechanical (substrate stiffness) and biochemical (proteins) cues on human muscle stem cell behaviour to identify conditions that permit propagation to clinically relevant numbers. In addition, we will use transplantation studies into a new mouse model of DMD that matches the disease progression of human patients to establish the efficacy of a stem cell transplantation approach to reverse the deleterious effects of progressive skeletal muscle wasting. The proposed studies promise to substantially increase our knowledge of human muscle stem cell regulation and potentiate development of novel therapeutic approaches to treat DMD patients.

Lorene Marie Nelson Ph.D. epidemiology

RG	Gene-Environment Interactions in the Etiology of ALS			
	\$93,494.00	1/1/2011	3/31/2013	Year 3

Summary Only 5-10% of ALS patients have a familial (genetic) form of the disease. Among the remaining 90% of patients with sporadic ALS, the cause is unknown. It is likely that a multifactorial process causes ALS, with contributions from both environmental and genetic factors. Using data from a recently completed epidemiologic study of ALS, we will investigate whether exposure to metals or pesticide chemicals is associated with the risk of developing ALS, and whether certain genetic factors either increase or decrease the risk associated with these exposures. By determining whether genetic factors modify the risk associated with these environmental agents, we hope to provide insight regarding the biological basis for the development of ALS. If these factors are shown to play a role in the cause of ALS, this will contribute to knowledge about the mechanisms of disease. With this knowledge, strategies could be developed to prevent ALS or to slow disease progression among affected individuals.

Lawrence Steinman M.D.

RG	Immune Tolerance to AAV and Dystrophin for Gene Therapy				
	\$102,687.00	2/1/2012	1/31/2013	Year 1	
	\$102,687.00	2/1/2013	1/31/2014	Year 2	
	\$102,687.00	2/1/2014	1/31/2015	Year 3	

Summary We will explore how we might tolerize to AAV and to immunogenic domains of dystrophin, in order to overcome two problems in future gene therapies for DMD: The immunogenicity of the vector and the immunogenicity of the dystrophin construct. Direct intramuscular injection of rAAV2 or rAAV6 in wild-type dogs resulted in robust T-cell responses to viral capsid proteins, and others have shown that cellular immunity to adeno-associated virus (AAV) capsid proteins coincided with liver toxicity and elimination of transgene expression in a human trial of hemophilia B. We have developed a technology with an engineered DNA vaccine to induce tolerance to self-proteins and to foreign proteins. Successful pre-clinical and human clinical trials have been taken forward in MS and Type 1 Diabetes with this approach. We now plan to extend it to DMD, and to attempt to tolerize to AAV and to dystrophin.

San Diego - San Diego State University Research Foundation Sanford I. Bernstein Ph.D.

RG	Disease mechanism	and therapy development f	or inclusion body	myopathy type 3
	\$123,437.00	2/1/2012	1/31/2013	Year 1

\$123,437.00	2/1/2013	1/31/2014	Year 2
\$123.437.00	2/1/2014	1/31/2015	Year 3

Dominant hereditary inclusion body myopathy type 3 (IBM-3) is caused by a mutation in Summary myosin, the molecular motor that drives muscle contraction. We will exploit an IBM-3 model that we developed in Drosophila (fruit flies) to define the cellular basis of this disease and to test potential therapies. Our biochemical and ultrastructural studies showed that IBM-3 myosin is prone to unfolding and aggregation. Further, muscle appears to respond to the mutant protein by producing autophagosomes, cellular bodies designed to encapsulate and degrade protein aggregates. We will test the hypothesis that the mutant myosin is labeled by addition of ubiquitin peptides and that the ubiquitin-tagged myosin is deposited in autophagosomes for degradation. We will use immunological and biochemical approaches to delineate the components of IBM-3 protein aggregates and examine whether they include proteins of the autophagosome, the proteasome (another cellular degradation organelle), the protein folding machinery and/or the aging program. Finally, we will incorporate the knowledge gleaned from these studies to test pharmacological and gene inhibition/enhancement approaches designed to hasten the clearance of protein aggregates and/or ameliorate defective muscle structure and function in IBM-3. This will be relevant to other aggregate-inducing diseases like nemaline myopathy and inclusion body myositis.

San Francisco - California Pacific Medical Center Robert G Miller MD

RG	Infrastructure for a Small Screening Trial Consortium - MDA ALS CRNG					
	\$306,000.00	1/1/2013	12/31/2013	Year 2		
	\$306.000.00	1/1/2014	12/31/2014	Year 3		

Summary The missions of the MDA/ALS Clinical Research Network (CRN) are to create productive and meaningful collaborations among the 5 major ALS clinical research centers, to standardize and optimize clinical care for regional ALS centers, and to promote collaborative research efforts among all ALS clinics. The enhanced communication and collaboration among MDA clinics will improve the care provided to ALS patients and families by promoting development of standardized care practices and quality measures, and enhancing referral and recruitment for clinical trials and studies. By supporting these missions, the CRN creates the infrastructure for the performance of high impact clinical research in ALS.

San Francisco - The Regents of the University of California, San Francisco (Contracts & Grants) Eric Jinsheng Huang M.D., Ph.D.

RG	Mechanisms of FUS mutations in motor neuron survival and synaptogenesis				
	\$137,500.00	2/1/2012	1/31/2013	Year 1	
	\$137,500.00	2/1/2013	1/31/2014	Year 2	
	\$137.500.00	2/1/2014	1/31/2015	Year 3	

Summary ALS is caused by the selective degeneration of motor neurons in the central nervous system. Patients with ALS suffer from severe muscle wasting/weakness and eventually die from respiratory failure. Recent genetic data have shown that mutations in the FUS gene can be identified in more than 5% of patients with familial ALS. One important pathological feature in familial ALS with FUS mutations is the presence of abnormal protein aggregates in motor neurons prior to their degeneration. However, it is unclear if abnormal protein aggregates directly contribute to the degeneration of the motor neurons. Based on the function of FUS as a RNA binding protein, we hypothesize that mutation in FUS interferes with normal RNA/protein synthesis, which ultimately leads to cell death and the degeneration of motor neuron synapses. Our goal is to establish both cellular and transgenic mouse models to determine how mutant FUS proteins lead to neuronal cell death and the maintenance of the neuromuscular junction. In support of this view, our data indicate that spinal motor neurons in transgenic mice expressing mutant FUS proteins show severe disruption in RNA and protein synthesis machinery. These exciting results provide strong support that the models we established will provide

novel platforms to identify therapeutic targets to block cell death in motor neurons and to restore innervation at the neuromuscular junction.

Peter E Oishi MD

RG	Analysis of human muscle stem cells: Toward therapy for muscular dystrophies			
	\$180,000.00	2/1/2012	1/31/2013	Year 2
	\$180,000.00	2/1/2013	1/31/2014	Year 3

Summary The muscle weakness experienced by those affected by muscular dystrophies is thought to occur when the normally occurring adult stem cells that repair damaged muscle are prematurely exhausted by regular activity. We will use human induced pluripotent stem cells to generate patient-specific muscle cells to repair the damaged muscle that accumulates in muscular dystrophies.

COLORADO

Aurora - University of Colorado Denver, AMC and DC Kurt Beam Ph.D.

RG Voltage sensor for excitation-contraction coupling

\$101,146.00 7/1/2012 6/30/2013 Year 3

Summary In response to input from the nervous system, an electrical impulse is produced in skeletal muscle cells, which in turn causes muscle contraction. This process, termed excitation-contraction coupling, is known to depend on two muscle proteins, the dihydropyridine receptor (DHPR) and type 1 ryanodine receptor (RyR1), but the molecular mechanism is not understood. The goal of this research is to identify the portions of the DHPR which "sense" the electrical impulse. This research will provide information that is currently lacking about an essential function in skeletal muscle, and provide new insights into human muscle diseases, including periodic paralyses and central core disease, which arise from mutations of the DHPR and RyR1.

Kurt Beam Ph.D.

RG	Analyzing DHPR-RyR1 interactions in a reduced system			
	\$100,000.00	8/1/2013	7/31/2014	Year 1
	\$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	2/1/2013	1/31/2014	Year 1	
	\$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.

Bradley Olwin Ph.D.

RG Identification and Characterization of Satellite Stem Cells

\$123,055.00	2/1/2012	1/31/2013	Year 2
\$123,055.00	2/1/2013	1/31/2014	Year 3

Summary Diseases that result in progressive loss of skeletal muscle tissue indicate that the normal regenerative processes in muscle are disrupted and are not capable of sustaining normal repair. If the cells responsible for normal repair could be augmented by drug therapies or cell replacement therapies, this might enhance regeneration and slow or halt loss of skeletal muscle function. We have identified a rare stem cell that we believe is a primary source of skeletal muscle stem cells. Since we know little concerning these cells we are proposing experiments to understand where these cells come from, whether they are a primary contributor of muscle stem cells and if we can enhance muscle repair in mdx mice by cell transplantation of the satellite stem cells.

Fort Collins - Colorado State University Eric Ross Ph.D.

RG	Aggregation and toxicity of ALS- and IBM-associated prion-like domains				
	\$121,000.00	2/1/2013	1/31/2014	Year 1	
	\$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.

CONNECTICUT

Storrs - University of Connecticut David J Goldhamer Ph.D.

RG	Regulation of satellite cell lineage commitment in regeneration and disease				
	\$125,000.00	2/1/2012	1/31/2013	Year 2	
	\$125,000,00	2/1/2013	1/31/2014	Year 3	

Summary Satellite cells are muscle stem cells that are responsible for postnatal muscle growth and the repair of damaged muscle in injury and disease. In Duchenne muscular dystrophy, accumulation of intramuscular fat and connective tissue represent histological hallmarks of advanced disease, and this infiltration of non-muscle tissue significantly affects muscle structure and function. Satellite cells have been implicated as a possible cell of origin for the increased fat and fibrotic tissue, but their involvement remains uncertain and controversial. Using mouse lines developed in the lab that allow specific and permanent labeling of satellite cells, we will directly assess the contribution of satellite cells to fat and connective tissue infiltrates in mouse models of muscular dystrophy. MyoD and Myf-5 are key regulators of embryonic myogenesis that have been implicated in satellite cell functions. Using a new mutant allele of MyoD that will allow the timing of MyoD deletion to be controlled, we will investigate the requirement for MyoD and Myf-5 in myogenic commitment in vivo. Further, we will determine whether double mutant satellite cells can engraft into injured and dystrophic muscle, foundational data that will assess the potential utility of this cell type for therapeutic use.

DISTRICT OF COLUMBIA Washington - Children's Research Institute (CNMC) Sebahattin Cirak Ph.D

DG	Gene discovery of e	Gene discovery of exome-negative muscular dystrophy patients by nextgen RNAseq				
	\$60,000.00	2/1/2013	1/31/2014	Year 1		
	\$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.

Eric Hoffman Ph.D.

RG	Asynchronous remodeling: A force driving failed regeneration in DMD.				
	\$108,743.00	2/1/2013	1/31/2014	Year 1	
	\$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.

Kanneboyina Nagaraju Ph.D, D.V.M

RIG	Murine Preclinical Center for Neuromuscular Diseases (MPCNMD)				
	\$100,000.00	3/15/2012	3/14/2013	Year 1	
	\$100,000.00	3/15/2013	3/14/2014	Year 2	
	\$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.

Terence Anthony Partridge Ph.D.

RG	Measuring the dyna	mics of muscle growth a	, - ,	e model of DMD
	\$125,000.00	8/1/2012	7/31/2013	Year 2
	\$125,000.00	8/1/2013	7/31/2014	Year 3

Summary A crucial part of our ability to test for the efficacy of therapeutic agents and to understand their mode of action depends on the availability of convenient and cost-effective animal models. The main animal model for testing of potential therapies for Duchenne muscular dystrophy (DMD) is the mdx dystrophic mouse. This does not precisely reproduce the clinical picture of DMD in boys but it does manifest the major pathological features within the muscles. Thus, in both DMD boys and mdx mice, groups of muscle fibers degenerate, triggering both inflammation at the site and subsequent regeneration of replacement muscle fiber from the stem-cell-like satellite cells that normally inhabit the muscle fiber surface. Eventually these processes lose efficiency and the muscle is progressively replaced by scar tissue and fat. The main problem with this model is that we have only a crude understanding of the pathological processes and in particular that we possess no properly attested means of measuring these various processes in a growing mouse. This proposal is designed to provide a set of measures of the severity of the disease process so that we can properly assess the relative effectiveness of different therapeutic agents and can determine what part of the disease mechanisms they are affecting. As part of the validation, we will test three of the therapeutic that have been reported to have beneficial effects on the mdx mouse dystrophy.

Terence Anthony Partridge Ph.D.

RG	Role of satellite cells and pericytes in maintenance of dystrophic muscle				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

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	8/1/2013	7/31/2014	Year 1	
	\$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.

FLORIDA

Coral Gables - Miller School of Medicine of the University of Miami Ellen Fave Barrett Ph.D.

RG	Preserving motor nerve terminals in mouse models of familial ALS				
	\$99,034.00	2/1/2012	1/31/2013	Year 2	
	\$99,034.00	2/1/2013	1/31/2014	Year 3	

Summary Motor nerves end in motor nerve terminals (mnts) that convey the signal instructing muscles to contract. In mouse models of familial amyotrophic lateral sclerosis (fALS) and in at least some ALS patients, mnts degenerate before the death of their cell bodies in the spinal cord. Mnts might be especially susceptible to injury because they rely heavily on the calcium sequestration function of mitochondria, which is impaired in pre-symptomatic fALS mice and worsens with age. We predict that drugs that protect mitochondrial function will help preserve mnts in early symptomatic fALS mice. This prediction will be tested by infusing the test agent into one hind limb over a 4 week interval. Muscles in both hind limbs will then be analyzed to determine whether more mnts remain intact on the drugtreated side, and if so, whether or not those preserved mnts and their mitochondria remain functional. Localized, unilateral drug infusions will minimize complications that might arise with systemic drug application, and will increase our ability to distinguish between effective and ineffective drugs by comparing treated and control limbs in the same mouse. Experiments with fALS mice have demonstrated that preserving motor neuron cell bodies is not always sufficient to halt disease progression. A combination of mnt-preserving treatments identified by our study with other treatments that preserve motor neuron cell bodies might be effective in slowing disease progression in ALS.

Coral Gables - University of Miami

Michael Benatar Ph.D., M.D.

RG Pre-familial ALS (Pre-fALS) Study \$175,000.00 7/1/2012 6/30/2013 Year 3

Summary Treatment options for ALS patients remain limited despite decades of research. The reasons are many and complex, but are largely reflective of the many mysteries still held by this disease. With the exception of the genetic forms of ALS, the etiology remains unknown; the diagnosis is typically made late in the disease course, at which stage therapies may offer limited prospects for slowing or reversing disease progression. There is, therefore, an urgent need for biomarkers that might permit early diagnosis, monitoring disease progression, or tracking response to experimental therapy. The study of ALS prior to symptom onset, although a long-term undertaking, provides unique opportunities to elucidate many of the enigmatic aspects of this disease -- and we contend that such an approach is both essential and feasible. Asymptomatic individuals from familial ALS pedigrees, who are at risk for developing ALS based on their harboring a mutation in an ALS susceptibility gene, represent the only known population that could be studied prospectively. With funding from the MDA we initiated a study of this population ~2 years ago. In this follow-up project we will: (a) expand the current cohort; (b) provide uninterrupted follow-up of existing and new participants; (c) systematically evaluate a series of novel biomarkers; and (d) expand the Pre-fALS biospecimen repository for use by the ALS research community.

Gainesville - University of Florida Celine Baligand Ph.D.

DG	DG MRI/MRS evaluation of muscle function and treatment strategies in Pompe d			
	\$59,817.00	2/1/2012	1/31/2013	Year 1
	\$57,105.00	2/1/2013	1/31/2014	Year 2
	\$53,951.00	2/1/2014	1/31/2015	Year 3

Pompe disease is a rapidly progressive muscular dystrophy caused by a deficiency in *Summary* acid alpha-glucosidase (GAA), the enzyme responsible for the breakdown of glycogen into glucose within the cell. A mutation in Gaa results in the accumulation of glycogen in many organs including the liver, the heart and the skeletal muscles. Ultimately, Pompe disease leads to fatal heart disease and respiratory insufficiency. The current therapeutic approach, enzyme replacement therapy, has been successful in reducing cardiac involvement and improving survival rate; however, it requires bi-weekly systemic infusion of recombinant cell-derived GAA, and many patients eventually become in need of assisted ventilation. An alternative approach is gene therapy, which has the potential to correct gene expression with a single administration using adeno-associated virus. The pre-clinical optimization of the potential treatments and the evaluation of their efficacy and administration routes require appropriate tools of investigation in small animal models. We will develop and apply various magnetic resonance (MR) spectroscopy and imaging techniques at high magnetic field to non-invasively investigate the natural progression of the disease, including muscle glycogen content, vascular reactivity and mitochondrial capacity, and compare different treatments approaches in a transgenic mouse model (Gaa-/-).

Darin Falk PhD

DG	Treatment of Pompe Disease via Retrograde Transduction of AAV Vectors				
	\$59,996.00	2/1/2012	1/31/2013	Year 1	
	\$59,925.00	2/1/2013	1/31/2014	Year 2	
	\$59,925.00	2/1/2014	1/31/2015	Year 3	

Summary Pompe disease is caused by a deficiency or absence of one enzyme that leads to an excessive accumulation of glycogen in the cell. Although once characterized as a heart and muscle disease, it is now known that Pompe disease displays complications similar to those caused by neuromuscular diseases. There is only one FDA approved treatment for Pompe and while outcomes are improved, the treatment still has limitations. Enzyme Replacement Therapy (ERT) must be administered at the clinic every other week for the patient's entire life and provides only a small elevation in enzyme activity resulting in incomplete clearance of glycogen. As a result, skeletal muscle weakness and respiratory complications evolve and vastly decrease the patient's quality and duration of life. This is further complicated by the inability of ERT to clear glycogen in the central nervous system which we believe has serious consequences. Our recent work demonstrates that reducing glycogen storage within the central nervous system alone provides significant improvement in breathing. To effectively address this disease, we will use a gene therapy approach to treat both skeletal muscle and the central nervous system simultaneously. Our strategy for developing a treatment for Pompe disease has three important features: 1) targeted and robust gene replacement; 2) long-term and continuous therapeutic efficacy; and 3) safe expression and delivery.

Sean Forbes Ph.D.

DG MRI/MRS assessment of perfusion and metabolism in dystrophic muscle \$59,499.00 7/1/2012 6/30/2013 Year 3

Summary Duchenne muscular dystrophy (DMD) is characterized by progressive muscle weakness, deteriorating functional capabilities, loss of independence, and early death. Muscles in children with DMD are deficient in dystrophin, which is accompanied by a lack of sarcolemma-localized neuronal nitric oxide synthase (nNOS). This loss of nNOS results in reduced local blood flow that may lead to muscle damage. The overall hypothesis of this project is that DMD is characterized by impaired vascular control during muscle contractions, leading to disruption in the normal coupling between oxygen delivery and muscle metabolism. It is expected that this reduction in muscle blood flow will result in metabolic perturbations in the muscle and augment damage. Furthermore, we anticipate that improving muscle blood flow will lessen damage in dystrophic muscle. To test these hypotheses, novel magnetic resonance imaging (MRI) and spectroscopy (MRS) methods will be implemented to measure high-resolution changes in muscle perfusion, metabolism, and damage in mouse models. These non-invasive techniques may prove to be sensitive tools to monitor and visualize disease progression and effectiveness of treatment in DMD.

Laura P.W. Ranum Ph.D.,

RG	Molecular Effects of Repeat Associated Non-ATG Translation in Myotonic					
	Dystrophy					
	\$138,364.00	2/1/2012	1/31/2013	Year 1		
	\$138,364.00	2/1/2013	1/31/2014	Year 2		
	\$138,364.00	2/1/2014	1/31/2015	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	8/1/2013	7/31/2014	Year 1	
	\$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	9th International Myotonic Dystrophy Consortium Conference			
	\$8,000.00	10/16/2013	10/19/2013	Year 1

Summary The International Myotonic Dystrophy Consortium Conference (IDMC) is the premier meeting in the DM field since it brings together such a diverse audience of basic and clinical research scientists, clinicians, DM families and caregivers. Many attendees are involved in research that investigates the pathomechanisms of DM in animal models and in vitro cell systems. Others are clinicians involved in therapeutic trials, the development of standards of care, and the development of new treatment strategies. Young scientists are also important contributors at IDMC, and past funding from the MDA has supported these new investigators, including post-doctoral fellows, medical students, and assistant professors. Many of these young investigators would have been unable to attend the conference without this support. A significant aspect of this meeting is that the afternoon of the final meeting day is reserved for a lunch and discussion between scientists, DM families and caregivers, advocacy groups as well as funding and regulatory agencies. Special sessions are devoted to patient care and outcome measures and IDMC-9 will build upon the success of previous IDMC conferences to promote multidisciplinary discussions and a congenial atmosphere.

Miami - University of Miami School of Medicine

Antoni Barrientos Ph.D.

RG	Role of New Evolutionary Conserved Cytochrome c Oxidase Assembly Chaperones				
	\$115,500.00	2/1/2012	1/31/2013	Year 2	
	\$115,500.00	2/1/2013	1/31/2014	Year 3	

Cytochrome c oxidase (COX) deficiency is the most frequent cause of mitochondrial *Summary* neuromyopathies in humans which as a whole have an incidence of 1:5,000. Patients afflicted with these diseases present heterogeneous clinical phenotypes, including Leigh syndrome, muscle weakness and encephalomyopathy. A better understanding of COX biogenesis is essential for elucidating the molecular basis underlying this group of diseases. In most patients suffering from mitochondrial disorders associated with COX deficiency, the molecular basis of their disease remains unknown. We have recently identified human homologues of two catalytic subunit-specific COX assembly chaperones previously identified in yeast. Their characterization is expected to provide significant information concerning mitochondrial translation of COX subunits in humans as well as on the process of their assembly into the enzyme. The genes encoding these conserved chaperones represent novel candidates when screening patients for mutations associated with COX deficiency. The main objective of the proposed research is to investigate their role in COX assembly using the yeast Saccharomyces cerevisiae and human cultured cells as research models. Our long-term goal is to attain a complete understanding of the pathways leading to COX assembly and their components as a prerequisite to the development of therapies for the management of disorders associated with COX deficiencies.

Carlos T. Moraes Ph.D.

RG	Increased Mitochondrial Biogenesis as Therapy to Mitochondrial Disorders				
	\$121,224.00	7/1/2012	6/30/2013	Year 3	

Summary Muscle degeneration is a hallmark of several neuromuscular disorders. We have recently shown that by increasing mitochondrial biogenesis, we can delay the onset of a mitochondrial myopathy and sarcopenia (age-associated muscle degeneration). We now propose to better study this compensatory mechanism by determining the time and duration of an increase in the expression of a gene that controls mitochondrial biogenesis (PGC-1alpha) for these beneficial effects to a mouse model of mitochondrial myopathy. We will also determine whether drugs that induce mitochondrial biogenesis (without or with concomitant endurance exercise) can also improve the myopathy.

Stephan Zuchner M.D.

RG	Gene identification in axonal CMT families				
	\$130,000.00	8/1/2012	7/31/2013	Year 1	
	\$130,000.00	8/1/2013	7/31/2014	Year 2	
	\$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 PABP	ABPN1: Implications for Oculopharyngeal Muscular Dystrophy			
	\$60,000.00	2/1/2013	1/31/2014	Year 1	
	\$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	2/1/2013	1/31/2014	Year 1	
	\$135,000.00	2/1/2014	1/31/2015	Year 2	
	\$135,000.00	2/1/2015	1/31/2016	Year 3	

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	Models and Treatments for Motor Neuron Disease			
	\$79,362,80	12/1/2012	11/30/2013	Year 1

Summary One of the earliest pathological features of ALS and other motor neuron diseases is the retraction of the motor nerve fibers from the muscle, leading to weakness and eventually to death. The cause of this phenomenon is unknown. Our previous work showed that in the superoxide dismutase 1 (SOD1) knockout mouse, an animal lacking the major enzyme to combat oxidative stress, SOD1, the motor nerve fibers retract as they do in models of ALS. Replacing SOD1 only in the mitochondria, which are energy producing organelles within the cell, completely protects against this pathology. Here we propose to create a new genetic mouse model where mitochondria will be "driven" down the nerve fiber by the introduction of Miro, a molecule that attaches mitochondria to their transport mechanism. We hypothesize that increasing the numbers of mitochondria at the connection site between the nerve and muscle will protect against nerve fiber retraction in the SOD1 knockout mouse, and in the mouse model of ALS. If successful, this work will lead to new strategies for protecting the nerve-muscle interface in motor neuron diseases.

Madhuri R Hegde B.S, M.S, Ph.D

RG	A comprehensive approach to identifying novel genes associated with NMDs			
	\$131,464.00	8/1/2012	7/31/2013	Year 1
	\$131,464.00	8/1/2013	7/31/2014	Year 2
	\$.00	8/1/2014	7/31/2015	Year 3

Summary A comprehensive approach to identifying the causative gene and understanding the underlying mechanism associated with each disease genotype is inevitable to diagnose disease and eventual selection of effective therapeutic strategy. In this project we will screen a large set of patient samples with known and unknown forms of muscular dystrophies by whole exome sequencing and targeted array analysis to identify the causative gene and the associated genotypes. For each identified candidate gene and genotype, we will later perform confirmation studies by transcript expression analysis (qRT-PCR) and western blot analysis to understand the nature of substantial alterations in the expression patterns of the muscle proteins. Mutations in a single gene and altered levels of the corresponding protein may further alter the expression pattern of closely related proteins, especially in the case of muscle proteome where several proteins form structural complexes, thereby modifying of the severity the disease phenotype and showing overlapping features. By our comprehensive approach

and confirmation studies we will better delineate the disease subtypes. Identification of novel genes and disease delineation will help effective disease diagnosis and choose appropriate therapeutic approach.

Madhuri R Hegde B.S, M.S, Ph.D

RG A cost effective approach for newborn screening for DMD

\$84,924.00 2/1/2013 1/31/2014 Year 1

Summary DMD is generally diagnosed by the age of five and advances with age. Even though early detection and diagnosis may not significantly change the course of intervention, recent study conducted by Centers for Disease control (CDC) and prevention has suggested that early diagnosis could have several advantages to the family. This could involve planning of further children or seeking genetic counseling for risk assessment and several others. Therefore, it is in the best interest of the patients, families and the nation to provide an efficient newborn screening method for DMD to reduce further burden. Our proposed study will help establish a high-throughput, low cost screening method which can be implemented in NBS programs to efficiently rule out the false positives detected by the CK level testing. This will allow for a successful two-tier screening method, involving initial CK level testing and immediate genetic testing for any positive cases, using the same blood spot card. The project is aimed to ultimately reduce DMD disease burden.

Grace Pavlath Ph.D.

RG	Mechanisms of Myofiber Branching				
	\$98,423.00	2/1/2012	1/31/2013	Year 2	
	\$98.423.00	2/1/2013	1/31/2014	Year 3	

Summary Skeletal muscle is composed of myofibers, which are long cylindrical cells. When myofibers are injured they degenerate and subsequently regenerate from precursor cells. However, in many neuromuscular diseases muscle regeneration is aberrant, and myofibers acquire an abnormal morphology and contain numerous cellular "branches." Branched myofibers display various functional abnormalities and are also weaker and more prone to injury. In a mouse model of Duchenne Muscular Dystrophy, the major of myofibers become branched in adult mice. Although the exact contribution of branched myofibers to dystrophic muscle physiology is unclear, muscles containing high levels of branched myofibers are unlikely to function in a normal physiologic manner. Thus, decreasing the number of branched myofibers will likely be beneficial for improving muscle physiology. The mechanisms regulating myofiber branching are significant to elucidate from both a basic and a clinical standpoint. We will study the role of two molecules in regulating myofiber branching during regeneration in mice. These experiments are novel because they are the first to mechanistically dissect regulation of myofiber branching in both wild type and dystrophic muscle. Potentially the molecular pathway examined in this proposal may be a good drug target in various neuromuscular disorders to prevent or reverse myofiber branching.

Grace Pavlath Ph.D.

RG A Knockin Mouse Model for Oculopharyngeal Muscular Dystrophy \$100,000.00 8/1/2013 7/31/2014 Year 1

Summary This is a study to characterize genetically engineered mice with the same change in their genes as people who suffer from oculopharyngeal muscular dystrophy. These mice will be the first opportunity to accurately model this disease in mice and could provide a tool both for understanding how the disease affects muscle and for finding therapies to treat the disease.

Wilfried Rossoll Ph.D.

RG The role of the ALS disease protein TDP-43 in motor neurons \$119,551.00 7/1/2012 12/31/2013 Year 3

Summary Recently, aggregation of a little studied protein called TDP-43 has been identified as a hallmark of the majority of amyotrophic lateral sclerosis (ALS) cases. Most studies on the role of TDP-43 protein have used non-neuronal cell lines but its function in motor neurons remains unclear. We will use primary motor neurons and motor neurons generated from stem cells as a tool to study the function of TDP-43 in this cell type, and to gain information about its involvement in ALS. Understanding the function of TDP-43 in motor neurons will help us to better understand the disease mechanism of ALS

and related neurodegenerative disorders. As a long term objective, this knowledge may help us to develop novel therapies to delay or even preventing motor neuron degeneration in patients suffering from ALS.

Augusta - Georgia Regents University Lin Mei M.D./Ph.D.

RG	Mechanisms of LRP4 autoantibodies in myasthenia gravis				
	\$130,000.00	8/1/2012	7/31/2013	Year 1	
	\$130,000.00	8/1/2013	7/31/2014	Year 2	
	\$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

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

RG	SMN inductive therapy in mild SMA				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	
	\$135,000.00	2/1/2014	1/31/2015	Year 2	
	\$135,000.00	2/1/2015	1/31/2016	Year 3	

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 S	sics of Exon Skipped Dystrophin Rods				
	\$93,577.00	2/1/2012	1/31/2013	Year 1		
	\$85,837.00	2/1/2013	1/31/2014	Year 2		
	\$85,837.00	2/1/2014	1/31/2015	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 Matthew N. Meriggioli M.D.

ACTG GM-CSF in the therapy of autoimmune myasthenia gravis \$176,923.00 1/1/2013 12/31/2013 Year 3

Summary Autoimmune myasthenia gravis (MG) is a chronic disease in which the immune system targets the skeletal muscle acetylcholine receptor (AChR) causing symptoms of muscle weakness. Research in this proposal will build upon pre-clinical data from the applicant's laboratory, which have shown that treatment with a particular growth factor, GM-CSF, effectively suppresses MG in mice and does so by mobilizing "immune regulatory cells" that are AChR-specific. The research proposed in this application will specifically move this strategy closer to application to human MG by: 1)identifying the specific conditions required for GM-CSF's beneficial effects in mice, 2) investigating the importance and functional properties of immune regulatory cells in human MG, and 3) carrying out a pilot clinical trial of GM-CSF in MG patients with active symptoms. These studies may provide important information that will lead to a definitive, large-scale clinical trial of GM-CSF in autoimmune MG.

Muthusamy Thiruppathi Ph.D

DG	Defect in Immune Regulation in Myasthenia Gravis: Implications for Treatment.				
	\$60,000.00	2/1/2013	1/31/2014	Year 1	
	\$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

RG	Immunomodulation of Experimental Autoimmune Myasthenia Gravis				
	\$105,686.00	8/1/2012	7/31/2013	Year 1	
	\$105,686.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Hines - Chicago Association for Research & Education in Science Junping Xin Ph.D.

DG	Characterization of CD4+ T cell response in ALS mouse following nerve injury				
	\$60,000.00	8/1/2012	7/31/2013	Year 2	
	\$60.000.00	8/1/2013	7/31/2014	Year 3	

In ALS, the CNS is under surveillance by the immune system. A proinflammatory innate immune response rapidly seeks to neutralize perceived threats. A secondary anti-inflammatory response turns off the first to prevent collateral damage to surrounding tissue. Dysregulation of these responses can contribute to neurodegeneration, especially when shifted towards pro-inflammatory conditions that damage healthy tissue. CD4+ T cells, immune cells that regulate a variety of immune responses, play an important role in supporting motor neuron survival after nerve injury. CD4+ T cells are important in both delaying the onset of ALS and in decreasing mortality in ALS mouse models such as the SOD1 mouse. Understanding the protective mechanisms of CD4+ T cell-mediated neuroprotection would allow us to design new therapies. We therefore seek to answer the following questions. First, what subsets of CD4+ T cells develop in SOD1 mice after nerve injury? Second, are the neuroprotective properties of CD4+ T cells from SOD1 mice impaired? If so, is that reduction responsible for the increased motor neuron loss after nerve injury observed in SOD1 mice? These questions will be addressed in the current study; we anticipate that data from these studies will reveal important information for understanding the role of CD4+ T cells in ALS and for development of novel therapeutics.

Maywood - Loyola University Chicago, Health Sciences Division Renzhi Han Ph.D.

RG Efficacy of complement inhibition as a therapeutic strategy for dysferlinopathy \$135,000.00 7/1/2012 6/30/2013 Year 3

Summary A subset of muscular dystrophy referred to as dysferlinopathies are caused by mutations in the gene encoding dysferlin, a protein shown to play an important role in the membrane repair process in striated muscles. At present, no therapy exists for dysferlinopathies. Our long term goal is to design a therapy for dysferlinopathies. Loss of dysferlin in skeletal muscle results in prominent muscle inflammation and muscle wasting. However, questions remain concerning what causes muscle membrane injury and whether immunological attack plays an active role in muscle injury in the absence of dysferlin. Resolving these questions may provide clues for the development of effective treatment strategies. This project focuses on exploring the effect of interfering the complement system on the muscle pathology associated with dysferlin deficiency. The overall results of these experiments will advance our understanding of the pathological mechanism underlying dysferlinopathies. Future studies will use this information for the development of therapeutic strategies to treat dysferlinopathies.

Skokie - Les Turner ALS Foundation Maria Zeller M.A.

SG 8th Annual ALS/MND Nursing & Allied Healthcare Professionals Symposium \$2,500.00 11/7/2013 11/10/2013 Year

Summary The ALS/MND Nursing and Allied Healthcare Professionals Symposium brings together clinical coordinators, nurse clinicians, and other allied healthcare professionals who work directly with

ALS patients, providing them with practical and cutting edge information on the latest treatments and research.

INDIANA

Indianapolis - Indiana University (Indianapolis)

William Groh M.D.

RG Continued Follow-Up in the Registry of Arrhythmias in Myotonic Dystrophy Type 1

\$89,751.00 1/1/2012 6/30/2013 Year 3

Summary In 1997 we initiated recruitment into a clinical Registry of Arrhythmias in Myotonic pystrophy at 23 MDA clinics throughout the U.S. to gather medical, genetic, and heart data on patient

Dystrophy at 23 MDA clinics throughout the U.S. to gather medical, genetic, and heart data on patients with the neuromuscular disease, myotonic dystrophy type 1. We enrolled 406 patients with myotonic dystrophy type 1. Although our study was initiated to look primarily at heart issues, our careful collection of patient information has allowed us to understand better the lives of those affected by DM1. Our current goal is continue monitoring these patients to determine their overall outcomes. Our data will provide a knowledge base to assess and compare outcomes of current practices to future interventions or therapies in the myotonic dystrophy population.

Ronald Mark Payne M.D.

RG Mechanism of Heart Failure in Friedreich Ataxia

Summary Friedreich Ataxia is the most common ataxia in humans and affects multiple organ systems. In particular, the heart is badly affected and patients die from a severe cardiomyopathy and heart failure. There is no cure. We have made a discovery that may explain why these hearts fail, and how we can prevent this from happening. The goal of this project is to understand the basic mechanism of heart failure in this disease, and then develop approaches to improve heart function and save lives.

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	8/1/2012	7/31/2013	Year 1	
	\$125,000.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Jennifer Rachel Levy Ph.D.

DG	Pathways and consequences of non-dysferlin mediated membrane repair				
	\$60,000.00	8/1/2012	7/31/2013	Year 2	
	\$60,000,00	8/1/2013	7/31/2014	Year 3	

Summary All cells have a plasma membrane that separates the intracellular content from the extracellular space. When the plasma membrane is damaged, it relies on molecular repair mechanisms to patch the sites of injury and prevent leakage of this content. Muscle-cell membranes undergo particularly frequent rounds of damage and repair due to exercise-associated plasma-membrane rupture. This repair is ineffective in dysferlinopathies, a class of muscular dystrophies caused by a defect in the protein dysferlin, which is a key player in the membrane repair process. Studies of mouse models of the dysferlinopathies have shown that plasma-membrane resealing in response to damage is defective in this context. However, muscle damage in dysferlinopathy is accompanied by inflammation,

and the relationship between defects in dyferlin-mediated repair and this inflammation is not fully understood. In this project, I aim to identify the aberrant membrane repair mechanisms that compensate for loss of dysferlin in dysferlinopathy patients. Further, I seek to determine if dysferlin-independent membrane repair signals immune cells to augment inflammation at sites of muscle injury. Identifying new factors that contribute to muscle inflammation in dysferlinopathy patients is expected to lead to the discovery of new therapeutic strategies for additional muscular dystrophies that are also associated with inflammation.

John Daniel Lueck Ph.D.

DG	Pathophysiology of Muscle Weakness and Wasting in Myotonic Dystrophy				
	\$60,000.00	2/1/2012	1/31/2013	Year 2	
	\$60,000.00	2/1/2013	1/31/2014	Year 3	

Summary The hallmark clinical manifestations of myotonic dystrophy are insulin resistance, muscle weakness and wasting, and myotonia. Since the discovery of the genetic misstep that results in myotonic dystrophy type 1 (DM1), several studies have increased our understanding of the rather unique and complex mechanism of the disease. In support of the multisystem pathology, the genetic flaw affects the expression and function of several proteins. Using DM1 mouse models and tissue from DM1 patients, advancements have been made in understanding the mechanism behind both myotonia and insulin resistance. In contrast, very little is known about the mechanism behind progressive muscle weakness and wasting. Our preliminary results suggest that dysregulated expression and splicing of genes important for maintaining proper intracellular calcium levels and membrane integrity may underlie muscle pathology. To determine how the disruption of specific genes contribute to muscle weakness and wasting, we will use a multifaceted approach to analyze DM1 mouse models and tissue from DM1 patients. Results from these studies will not only shed light on the mechanism of muscle disease in DM1, but will also help to illuminate the disease pathways of other muscular dystrophies. Ultimately, the goal of this research is to discover therapeutic strategies for DM1.

Michael Shy M.D.

RIG	North American CMT Network				
	\$.00	12/31/2012	12/31/2013	Year 3	

Summary The CMT North American Database currently includes a large number of well studied patients with different types of CMT to be available for clinical trials and clinical investigations. To improve the Database, ensure that patients are evaluated in a uniform fashion and to provide an infrastructure that will lead to high quality research for patients throughout the United States we are extending the Database and creating the North American CMT Network. Patients within the Network will be evaluated at one of six Centers of Excellence throughout the United States, DNA samples will be banked, and scoring systems for children with CMT will be established. This CMT Network will provide the infrastructure for CMT research within the United States and throughout the world.

Michael Shy M.D.

RIG	North American Charcot-Marie-Tooth (CMT) Consortium			
	\$145,043.00	1/1/2013	12/31/2013	Year 1
	\$145,917.00	1/1/2014	12/31/2014	Year 2
	\$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.

KENTUCKY

Louisville - University of Louisville Research Foundation, Inc. Ashok Kumar Ph.D.

RG Therapeutic Targeting of Matrix Metalloproteinases in Muscular Dystrophy \$116,402.00 7/1/2012 6/30/2013 Year 3

Summary Matrix Metalloproteinases (MMPs) are a group of extracellular proteolytic enzymes linked to extracellular matrix remodeling and pathogenesis in several diseases involving tissue destruction. However, the role of MMPs in skeletal muscle pathogenesis in Duchenne Muscular Dystrophy (DMD) is less clear. Ongoing studies in our laboratory have provided strong evidence that the expression of several MMPs is drastically increased in dystrophic muscle of mdx mice (a mouse model of DMD). We have also obtained initial evidence that the inhibition of MMPs can attenuate myopathy in mdx mice. In this project, we will rigorously investigate whether pharmacological inhibitors of MMPs can reduce skeletal muscle pathogenesis in animal models of DMD. We will also investigate the mechanisms by which the increased expression of MMPs causes muscle loss in these models of mice. This study should provide a base for undertaking further investigations in humans whether MMPs can be used as targets to attenuate disease progression in DMD patients.

MARYLAND

Baltimore - Hugo W. Moser Research Institute at Kennedy Krieger, Inc. Kathryn R. Wagner M.D., Ph.D.

RG Myostatin Regulates Fate of Satellite Cells in Dystrophic Muscle \$117,513.00 8/1/2012 7/31/2013 Year 2 \$117,513.00 8/1/2013 7/31/2014 Year 3

Summary In most muscular dystrophies and chronic myopathies, muscle regeneration becomes less effective over time and muscle is replace by fibrosis or scar tissue. The factors that govern establishment of fibrosis are not well understood. However, myostatin, a regulator of muscle growth, is one important factor in development of fibrosis. In the absence of myostatin, muscle regenerates more quickly and with less fibrosis. The studies described in this grant application will determine whether one of the cells that is important for muscle regeneration, the satellite cell, can become misdirected to contribute to muscle fibrosis. The studies will specifically evaluate whether myostatin is a cue that directs satellite cells away from forming new muscle and toward fibrosis. If this hypothesis is correct, then anti-myostatin therapies, currently in clinical trials for muscular dystrophy, will have an important role to play in stimulating muscle regeneration and reducing muscle fibrosis in a variety of clinical scenarios.

Kathryn R. Wagner M.D., Ph.D.

SG MDA/AFM Joint Evening Symposium \$8,000.00 5/15/2013 5/18/2013 Year 1

The ASGCT Annual Meeting serves as the only conference within the United States that Summary provides a comprehensive forum for presentation of issues specific to the development of genetic and cellular therapies for rare inherited and acquired diseases. The Annual Meeting will educate physicians and researchers on basic science issues, the status of investigational clinical trials, and information about regulatory and compliance issues. The ASGCT Annual Meeting consists of 10 education sessions, 24 scientific symposia, over 20 oral abstract sessions and daily poster abstract sessions. The Education Sessions are generally Topical Reviews in a specific area or Emerging Field Reviews for basic research than is just entering clinical investigations. Each Scientific Symposium is approximately two hours and covers an area of significant advancement. Three to four speakers conducting leading research in the chosen area are invited. Oral abstract presentations are chosen by peer review from a total of 22 committees comprised of leaders in the field. Presentations are 10 minutes in length with 5 minutes for questions. In addition the main sessions, ASGCT partners with related foundations to host evening sessions focused on the topic most relevant to the partnering foundation. The evening sessions offer the foundations the unique opportunity to explore the most recent scientific and clinical advancements critical to a particular indication of interest as well as investigate areas in most need of additional

research. The symposia also offer foundations the opportunity to address a crowd of world renowned gene and cell therapists while promoting their cause to post-doctoral fellows and trainees in search of their own research focus.

Baltimore - Johns Hopkins University School of Medicine Elizabeth H. Chen Ph.D.

RG	Functional analysis of the small GTPase Rho1 in myoblast fusion in vivo					
	\$107,163.00	2/1/2012	1/31/2013	Year 1		
	\$107,163.00	2/1/2013	1/31/2014	Year 2		
	\$107.163.00	2/1/2014	1/31/2015	Year 3		

Summary Skeletal muscle is a unique organ that is composed of multinucleate muscle fibers, resulting from fusion of hundreds or even thousands of mononucleate myoblasts. Myoblast fusion is not only required for myogenesis during embryogenesis, but is also critical for postnatal muscle growth, maintenance and regeneration. In response to damaged or myopathic skeletal muscle, the normally quiescent adult muscle stem cells (satellite cells) become activated, proliferate and differentiate to form fusion-competent myoblasts, which then fuse with existing myofibers or with one another to fully regenerate the muscle. The fusogenic capacity of myoblasts has also been exploited in cell-based therapy using skeletal muscle as the prime organ for gene delivery. Thus elucidating the mechanisms underlying myoblast fusion will not only contribute to our understanding of skeletal muscle biology, but also lead to improvements in the efficacy of muscle regeneration in the treatment of a broad range of muscle degenerative diseases. This project will investigate the function of a new regulatory factor required for myoblast fusion. The mechanistic understanding of the function of this new gene will expand our knowledge of myoblast fusion and ultimately lead to a positive modulation of myoblast fusion efficiency in the treatment of muscle degenerative diseases.

Mohamed H. Farah PhD

RG	Enhancing neuromuscular reinnervation by BACE1 inhibition				
	\$125,000.00	2/1/2013	1/31/2014	Year 1	
	\$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.

David A. Kass MD

RG Protein Kinase G inhibition of TRPC to Benefit the Dystrophin-deficient Heart \$105,309.00 2/1/2012 1/31/2013 Year 2

Summary Duchenne muscular dystrophy results from a genetic lack of the protein dystrophin, which leads to progressive and severe skeletal and heart muscle weakness. This gene defect also results in depression of the function of the enzyme nitric oxide synthase (NOS), reducing localized levels of its primary downstream regulator, cyclic GMP and enzyme, protein kinase G. This is thought to generate a muscle nutrient supply/demand imbalance and contribute to muscle damage. Another feature of the disease is that ion channels located in the outer membrane, known as TRPC channels, become to be hyperactive, and this may increase calcium levels inside the cell that in turn can stimulate oxidative stress, and cause cell damage and/or death. We recently discovered that cyclic GMP dependent signaling can potently suppress TRPC channels directly. This suggests that treatments to increase cGMP levels such as sildenafil (Viagra), or activators of an enzyme called soluble guanylate cyclase, may be able to attack both the loss of normal NOS function, and hyperactive TRPC channels. The research we proposed in this grant will test the importance of cGMP/PKG activation to block stimulated TRPC

channels in experimental models of muscular dystrophy, and determine if this modification can inhibit muscle damage associated with excess calcium and oxidant stress. This work would enable us to move forward with clinical trials – as these are drugs already used to treat other human diseases.

Youngjin Lee Ph.D.

DG	Glial monocarboxylate transporters (MCTs) pathway in neurodegeneration				
	\$59,999.00	8/1/2012	7/31/2013	Year 2	
	\$59,999.00	8/1/2013	7/31/2014	Year 3	

One hypothesis for the neuronal death associated with ALS is that metabolically active neurons do not receive enough energy substrates from surrounding glia cells. An important energy substrate in the nervous system is lactate, which can be transferred into neurons via monocarboxylate transporters (MCTs) to support neuronal function. Interestingly, the expression of monocarboxylate transporter-1 (MCT-1), a lactate transporter, is significantly reduced in ALS patients and at disease endstage in SOD1 mutant mice, suggesting critical roles for MCT-1 in the pathogenesis of ALS. However, the expression, regulation, and function of MCTs, including MCT-1, in ALS are poorly understood. Therefore, we recently established MCT-1 and MCT-4 reporter mice to better understand MCT-1 expression in ALS. The analysis of double transgenic MCT-1 and MCT-4 reporter mice crossed with SOD1 animal models of ALS, as well as the use of MCT-1 heterozygote knockout mice, will give us great insights into the potential roles of MCT-1 and MCT-4 in neurodegeneration in ALS. The knowledge gained from these studies will provide novel therapeutic targets for preventing disease progression in ALS.

Thien Nguyen M.D., Ph.D.

RG	Axonal protection in Demyelinating Disorders			
	\$140,000.00	2/1/2012	1/31/2013	Year 2

\$140,000.00 2/1/2012 1/31/2013 1ear 2 \$140,000.00 2/1/2013 1/31/2014 Year 3

Summary The collective experience from demyelinating and dysmyelinating disorders such as Charcot-Marie-Tooth disease (CMT) suggests: (1) demyelination in itself can cause axonal degeneration, and (2) demyelination can also make axons more vulnerable to degeneration produced by inflammatory and other local stresses. This progressive axonal loss may account for much of the irreversible clinical deficits seen in patients. We hypothesize that demyelination is associated with loss of myelin-axon interactions and chronic defects in axonal support, leading to progressive axonal degeneration and increased susceptibility to axonal loss by local stresses. One candidate among the myelin components is netrin-1, which are present at the Schwann cell-axon interface of all myelinated internodes. Our preliminary data show that netrin-1 expression is markedly reduced by 3-4 folds in trembler-J (TrJ) mice, a model of human CMT1 disease. This suggests that netrin-1 may play an important role in axonal survival in CMT and may serve as a potential target for therapy. This project aims to investigate whether netrin-1 might protect axons in cell culture and ultimately, to test the potential therapeutic benefit of netrin-1 in a mouse model of CMT1. This would represent an important step in developing neuroprotective strategies to treat CMT.

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

RRG	Robert Packard Center for ALS Research (Wings 2011) (Rothstein, Jeffrey)				
	\$126.201.00	3/1/2012	2/28/2013	Year 1	

Summary MDA funding received (as directed by Wings Over Wall Street) will be used to help fund one (1) collaborative research project through the Robert Packard Center for ALS Research. 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 Advisors. Any additional funding required by 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.

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

RG	ALS C9ORF72 iPS cells: D	Development of an antisense-based therapy and biomarker		
	\$130,902.00	2/1/2013	1/31/2014	Year 1

\$130,902.00	2/1/2014	1/31/2015	Year 2
\$130.902.00	2/1/2015	1/31/2016	Year 3

Understanding the pathophysiology and development of new therapeutics for Summary 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 2012) (Rothstein, Jeffrey) \$121,376.18 8/1/2013 7/31/2014 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.

Shanthini Sockanathan PhD

RG Characterization of a new animal model for motor neuron degeneration \$113,864.00 7/1/2012 6/30/2013 Year 3

In order to prevent or cure motor neuron degenerative diseases, there is a pressing **Summary** need to identify the mechanisms involved in triggering degeneration, to define the pathways involved in preserving motor neuron survival, and to develop new animal models that facilitate the study and design of innovative treatments. We find that mice lacking the GDE2 protein (Gde2 nulls) initially have deficits in motor neuron production, but recover at birth to have normal numbers of motor neurons. Surprisingly, adult Gde2 nulls show dramatic motor neuron loss and remaining motor neurons appear to be dying. We hypothesize that loss of GDE2 leads to motor neuron degeneration, and that GDE2 may be a target in motor neuron degenerative disease. Here, we will characterize the neurodegenerative phenotype of Gde2 nulls in detail, perform a time course of analysis to define the onset and progression of motor neuron degeneration, and determine if all or subsets of motor neurons degenerate in the absence of GDE2. We will pair these analyses with behavioral studies to link the motor neuron pathology with motor function. Lastly, we will utilize mouse genetics to determine if GDE2 deficits in embryonic or adult motor neurons are responsible for motor neuron degeneration. This study will provide insight into pathways underlying motor neuron degeneration and will generate a new animal model for studying motor neuron degenerative processes.

Shanthini Sockanathan PhD

RG	The role of thiol-redox pathways in regulating motor neuron differentiation			
	\$132,000.00	2/1/2012	1/31/2013	Year 2
	\$132,000.00	2/1/2013	1/31/2014	Year 3

Summary In previous work, we showed that the six transmembrane protein GDE2 controls the production of spinal motor neurons through extracellular glycerophosphodiester phosphodiesterase

(GDPD) activity. Recently, we discovered that GDE2 activity is itself regulated by a second protein, Prdx1, which activates GDE2 by breakage of a disulfide bond that normally inhibits GDE2 function. Thus, the disulfide bond within GDE2 acts as a molecular switch that turns motor neuron production "on" or "off". This observation makes the novel discovery that thiol-redox pathways that modify disulfide bond formation play pivotal roles in the control of motor neuron differentiation. One question that emerges from this work is how are active forms of Prdx1 generated to ensure efficient initiation of GDE2-dependent motor neuron differentiation? Here, we will test the hypothesis that inactive forms of Prdx1 are reactivated through separate thiol-redox pathways. More recently, we find that another Prdx molecule is expressed with GDE2 and inhibits its activity, suggesting the possibility that this mechanism regulates the initiation, extent and rate of motor neuron differentiation. We will test this theory using a combination of embryological and biochemical approaches. Taken together, these studies will provide insight into how thiol-redox pathways intersect to control the onset and progression of motor neuron differentiation.

Charlotte Jane Sumner M.D.

RG	Characterization of TRPV4 associated peripheral neuropathy in animal models				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Baltimore - Johns Hopkins University, Bloomberg School of Public Health Jiou Wang M.D., Ph.D.

RG Elucidating the Molecular Mechanisms of ALS in			. elegans and Mammal	Mammalian Models	
	\$110,000.00	2/1/2012	1/31/2013	Year 2	
	\$110,000.00	2/1/2013	1/31/2014	Year 3	

Summary Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disease that bears the hallmark of the degeneration of motor neurons. Among the major genes linked to the classic forms of ALS are SOD1, TDP-43, and FUS. Research on SOD1, the first identified and most well-studied ALS gene, suggests that formation of protein clumps may play an important role in the pathogenesis of this form of disease. Interestingly, TDP-43 and FUS were recently found in abnormal protein clumps in ALS patients. However, since we know little about what these proteins normally do in the cell, it remains a mystery how they bring about the development of ALS. We recently demonstrated that a simple nematode (round worm), Caenorhabditis elegans, could be genetically engineered to model human ALS. The short-lived, transparent C. elegans is an ideal paradigm for dissecting the complex disease processes as well as testing potential therapeutic agents for neurodegenerative diseases. We plan to use the C. elegans models to identify genetic modifiers of the neurotoxicity of the ALS-associated proteins. The findings from C. elegans will be validated in mammalian cell systems and in mouse models. Using the combined C. elegans and mammalian systems, we hope to discover promising drug targets and therapeutic agents that can be quickly developed to combat ALS.

Baltimore - University of Maryland, Baltimore

Robert J. Bloch Ph.D., Harvard University, 1972

RG	Cellular and Molecular Studies of Dysferlinopathy				
	\$120,765.00	2/1/2012	1/31/2013	Year 1	
	\$120,765.00	2/1/2013	1/31/2014	Year 2	
	\$120,765.00	2/1/2014	1/31/2015	Year 3	

Summary Limb Girdle Muscular Dystrophy Type 2B (LGMD2B) and Miyoshi Myopathy are caused by mutations in the gene coding for the protein, dysferlin. Dysferlin is a large protein, but we have little information about what parts of it are important for its activity. We have found that most of the dysferlin in healthy muscle is associated with intracellular membranes that carry the electrical signal to initiate muscle contraction, but its role there is unknown. The work we propose will determine what parts of dysferlin are required for its function, and how the protein helps to stabilize the intracellular membranes of skeletal muscle. This information will be essential in designing and testing pharmacological or gene therapy approaches to treating muscular dystrophies linked to dysferlin.

Andrew Ziman Ph.D.

DG	Calcium Signaling in Dysferlinopathies				
	\$60,000.00	2/1/2013	1/31/2014	Year 1	
	\$60,000.00	2/1/2014	1/31/2015	Year 2	
	\$60,000.00	2/1/2015	1/31/2016	Year 3	

Dysferlinopathies are a family of recessive muscular dystrophies caused by mutations in Summarv the DYSF gene, which encodes the protein dysferlin. Clinical diagnosis of dysferlinopathies includes significant muscle weakness and biopsies showing signs of degeneration/regeneration, necrosis and inflammation. There are no therapies that slow the onset or progression of symptoms. While dysferlin has been associated with membrane repair mechanisms, little is actually known of its function in muscle. I hypothesize that dysferlin plays a significant role in maintaining the ability of muscle to function normally during and after muscle exercise and following injury. My results show that insults to muscle cells in tissue culture cause a rise in reactive oxygen (ROS) production and a disruption in Ca2+ regulation not seen in control fibers. Both can lead to pathogenic signals, including the activation of enzymes such as calpain, which cause death of the muscle fiber. Using this experimental paradigm, I will determine the dynamics of Ca2+ signaling and ROS activation in healthy and dysferlin-null muscle fibers. I will also test several prospective therapeutic compounds to learn if preventing the abnormal changes will preserve normal structure and function of dysferlin-null muscle. Finally, I will introduce either wild type or mutated forms of dysferlin in null muscle to determine which domains of the protein are necessary to convey protection from injury-induced pathology

Bethesda - Federation of American Societies for Experimental Biology Giovanni Manfredi M.D., Ph.D.

SG	Mitochondrial Biogenesis and Dynamics in Health, Disease and Aging			
	\$7.275.00	6/16/2013	6/21/2013	Year 1

Summary The FASEB Conference on Mitochondrial Assembly and Dynamics in Health, Disease and Aging will be held in June 2013 and is Co-organized by Dr. Geral Shadel (Yale University) and Dr. Giovanni Manfredi (Weill Cornell Medical College). Its goal is to promote the exchange of scientific ideas among basic and translational scientists interested in diverse aspects of mitochondrial biology and mitochondrial dysfunction in human neuromuscular diseases, with a focus on promoting collaborative approaches and cross-field stimulation. It will also encourage and promote participation by junior investigators, with the goal of integrating them in this important aspect of neuromuscular diseases. Recent scientific advances have led to the realization that the contribution of mitochondria to human neuromuscular diseases is multifaceted and more far-reaching than previously anticipated. Therefore, the relevance to the MDA mission is clear: There is a growing need to create interactions between investigators that understand and study the basic biology of the organelle with those engaged in studying diseases, with the goal of melding these activities into a better understanding of how

mitochondrial dysfunction contributes to human neuromuscular diseases. It is expected that there will be approximately 120 attendees at the meeting. Of these, 36 will be invited speakers and discussion leaders. In addition there will be registrants, including established investigators, postdocs, and students. Participants will present their work in the form selected platform presentations or posters.

Bethesda - National Institute on Aging, NIH NIA Bryan Traynor M.D., Ph.D.

RIG Exome sequencing of sporadic ALS: a public resource to accelerate gene discovery \$400,000.00 12/1/2012 12/1/2013 Year 1

Summary Driven by advances in sequencing technologies, genetic discoveries are revolutionizing how we think about ALS and other neurodegenerative diseases. We plan to further exploit the next generation sequencing capacity of our laboratory by exome sequencing a large cohort of sporadic ALS patients, and make these data publicly available online. This unique resource will accelerate the pace of genetic discovery by allowing researchers to rapidly replicate their findings and to estimate the frequency and relative importance of their newly discovered ALS genes. This project is feasible because of the promotional price offered by Illumina (\$360 per exome) and the sequencing resources available in our laboratory. The Intramural Research Program at the NIH will cover other expenses, thereby leveraging NIH resources to dramatically lower the overall cost of this infrastructure project.

Bethesda - Society for Muscle Biology Mary Baylies Ph.D.

SG Development, Function and Repair of the Muscle Cell

\$15,000.00 2/1/2012 1/31/2013 Year 1

This application requests partial support for the "Frontiers in Myogenesis" meeting, **Summary** "Development, Function and Repair of the Muscle Cell", sponsored by the Society for Muscle Biology. Our objective is to bring together experts in developmental, cellular and molecular biology, adult muscle biology, human genetics to stimulate muscle disease research and foster new collaborations at both the basic and clinical levels. Our meeting will be held at the Kimmel Center at New York University in New York City, on June 4-8, 2012. We expect over 300 participants from all over the world. Seven plenary sessions, presenting sixty-three speakers (20 selected from submitted abstracts) at the senior, midcareer and junior levels, will address: Origins, Patterning and Behaviors of Muscle Progenitors; Transcriptional Control of Myogenesis and Muscle Progenitors' Identity; Muscle Differentiation, Interaction of Muscle Cells with its Environment; Adult Muscle Progenitors, Satellite Cells: Specification and Contributions to Muscle Repair; Muscle Size Regulation; and Therapeutic Interventions to Human Muscle Disease. Three dedicated poster sessions will foster the exchange of ideas among all participants. A review of the meeting will be published in Skeletal Biology. This meeting will afford a comprehensive analysis and integration of recent discoveries and will emphasize how these advances inform our understanding of, and lead to potential therapies for, muscle disease.

MASSACHUSETTS

Boston - Beth Israel Deaconess Medical Center Zolt Arany M.D. Ph.D.

RG PGC-1 coactivators and angiogenesis in muscular dystrophy

\$116,978.00 7/1/2012 6/30/2013

Year 3

Summary It has become increasingly clear that skeletal muscle metabolism plays a critical role both in the onset of, and resistance to Duchenne and other muscular dystrophies. Our lab studies a small group of molecules, the PGC-1s, which powerfully regulate broad metabolic programs in various tissues. In skeletal muscle, these molecules markedly alleviate muscle degeneration in mdx mice, a model of DMD. How this happens remains unclear. Recently, we have shown that PGC-1s control a new and powerful pathway that induces the formation of new blood vessels in muscle. We therefore propose here the hypothesis that these molecules alleviate muscle degeneration and atrophy by

boosting microvascular density and signaling. The hypothesis will be investigated here using a variety of molecular and genetic means, using the mdx mouse as a model of DMD. Understanding precisely how PGC-1s protect skeletal muscle against the ravages of dystrophy may lead to novel therapeutic approaches for these devastating diseases.

${\bf Boston \hbox{-} Brigham \hbox{ and Women's Hospital, Inc.}}\\$

Steven A Greenberg MD

RG	The role of TDP-43 a	nd aberrant RNA metab	oolism in sIRM and hIRM	1
NO	\$150,000.00	2/1/2012	1/31/2013	Year 2
	\$150,000.00	2/1/2013	1/31/2014	Year 3

Summary Recent studies have identified visible redistribution of a protein TDP-43 from its normally nuclear location to the muscle sarcoplasm in approximately 25% of myofibers in inclusion body myositis(IBM)biopsy specimens. TDP-43 binds to RNA and it is likely that this redistribution has accounted for sequestration of important RNA molecules in muscle sarcoplasm. The goals of this project is to identify the consequences of sarcoplasmic redistribution of TDP-43 in muscle and determine the specific RNA molecules sequestered by it.

Xin Wang Ph.D.

RG	Screening Agonists of the Melatonin Receptor 1A against ALS			
	\$143 877 00	1/1/2012	6/30/2013	Year 3

Summary Melatonin receptor 1A (MT1) is depleted in apoptotic NSC34 motoneurons, in ALS transgenic mice, and in post mortem spinal samples from ALS patients, while administration of melatonin countered the loss of MT1. Our observations suggest that depletion of MT1 protein is not simply a consequence of ALS but actively drives disease progression. Moreover, the MT1 receptor is a potential drug target for the treatment of ALS. We aim to identify compounds that are therapeutic for ALS from agonists of the MT1 receptor based on the restoration of the target MT1. We will screen these agonists in NSC34 motoneurons and mSOD1G93A mice. This study should stimulate the search for more potent compounds and advance the pharmacological characterization of agonists of MT1. Thus the screening may help to develop novel therapeutic approaches to ALS.

Xin Wang Ph.D.

RG	Identifying 2-lodomelatonin and 8M-PDOT for ALS Therapy				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	
	\$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.

Matthew Alexander Ph.D.

DG	Role of miR-486 in the pathogenesis of Duchenne Muscular Dystrophy					
	\$60,000.00	2/1/2013	1/31/2014	Year 1		
	\$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.

Alan H. Beggs Ph.D.

RG	Molecular Genetics of Congenital Myopathies			
	\$133,978.00	8/1/2012	7/31/2013	Year 2
	\$129,034.00	8/1/2013	7/31/2014	Year 3

Summary The congenital myopathies are a diverse group of inherited neuromuscular conditions that result in skeletal muscle weakness of variable onset and severity. To better understand the causes of these disorders, which are commonly seen in MDA neuromuscular clinics, we are building an extensive registry and biorepository of cases and specimens from patients and their families. Simultaneously, we have initiated a mutation screen in zebrafish to identify novel lines of mutant fish with genetic mutations that lead to muscle defects similar to those seen in patients with congenital myopathies. We are now mapping and identifying many of these new zebrafish mutations, and have already discovered several to be in genes with known relationships to human neuromuscular diseases. In this project, we will map these new genes, and determine their nature and relationship to the muscle defects seen in the fish. This information will then be used to identify human patients and families with analogous muscle findings from our registry and from related and complementary registries belonging to collaborators. The relevant genes will be screened in these human cases to identify new human neuromuscular disease genes. Identification of these genes will provide the information necessary to develop accurate carrier and prenatal testing, and will hopefully lead to new insights into therapies for these conditions.

Emanuela Gussoni Ph.D

RG	Melanoma cell adhesion molecule (MCAM) in human myogenic cells				
	\$128,022.00	8/1/2012	7/31/2013	Year 2	
	\$128,022.00	8/1/2013	7/31/2014	Year 3	

Summary The direct injection of normal cells into mice and patients with muscular dystrophy has been attempted as a way to provide missing proteins, such as dystrophin, to the affected muscles. These studies have shown that, while the technique is safe, the muscles of patients do not show significant improvement. One of the causes for this inefficiency is the rapid death of the normal cells following injection. To overcome this problem, different cell types more capable of surviving the transplant and producing the desired protein must be identified. We will study new sub-fractions of human cells isolated based on the expression of the surface protein melanoma cell adhesion molecule (MCAM). We have preliminary evidence that MCAM-expressing cells are capable of forming muscle both in tissue culture and following injection into animals. We seek to determine: 1) whether cells expressing this

protein are pre-destined to become muscle, 2) how expression of MCAM protein is linked to myogenic potential and 3) whether fractions of human cells expressing MCAM can efficiently repair dystrophic muscle. These studies will help elucidate the function of MCAM in human muscle cells and determine whether MCAM-expressing cells are promising candidates for translational studies.

Peter B. Kang M.D.

RG Linkage analysis in limb-girdle muscular dystrophy

\$99,938.00 2/1/2012 1/31/2013 Year 2 \$99,914.00 2/1/2013 1/31/2014 Year 3

Summary At least 18 different genes have been linked to the Limb Girdle Muscular Dystrophies (LGMDs). Individually, each LGMD is rare, but collectively they form a major class of inherited myopathies. Identification of new genes that cause LGMD would help expand our knowledge of the muscular dystrophy disease process, and perhaps illuminate new therapeutic approaches for the muscular dystrophies in general. The candidate has accumulated and genotyped DNA samples from a number of families with LGMD. Known loci for LGMD have been excluded in 3 kindreds, raising the likelihood that novel genes are associated with the disease in these families. Mutations in known genes for LGMD have been identified in the other 14 families studied to date. The applicant will (1) continue to recruit new families with LGMD and perform linkage analysis; (2) identify potential mutations in novel genes using traditional and high-throughput sequencing technologies, as well as zebrafish morpholino suppression; and (3) determine which of the variants identified are likely to be causative mutations. This project has the potential to yield tangible and useful scientific knowledge in the study of muscular dystrophies.

Louis Kunkel Ph.D.

RG Small Molecule Screens in Dystrophin Deficient Zebrafish \$125,000.00 7/1/2012 6/30/2013 Year 3

Summary Zebrafish are an excellent animal model of human disease on which to develop possible therapies. They can be obtained in large numbers, they are small, transparent early in life and are permeable to small molecules. There are two genetic models of dystrophin deficiency in zebrafish, one caused by a stop code in exon 4 and one which a 5'splice site mutation at the end of exon 62 which removes this exon from the transcript. Both fish have a severe skeletal muscle phenotype which results in death of most fish by 10 days post fertilization and can be detected as early as 3 days post fertilization using birefringence under polarized light. We have preformed a preliminary screen of one of three chemical libraries of drugs currently approved for use in humans. We have identified 7 compounds which increase the survival rate of these dystrophin deficient fish from a 10% survival rate to as much as 60% survival. Our preliminary data indicate that this substantial increase in survival is observed in both alleles of dystrophin deficiency and thus are not correcting the dystrophin mutation. Each of these molecules is an excellent candidate to modulate disease progression in human muscular dystrophy. The project aims not only identify additional compounds but to also look at the targets and mechanism of action for these compounds by their analysis in zebrafish and mice.

Jianming Liu Ph.D

DG Targeting Smad mediated signaling of TGFbeta family for stem cell therapy of DMD \$59,929.00 2/1/2012 1/31/2013 Year 2 \$59,997.00 2/1/2013 1/31/2014 Year 3

Summary A hallmark of Duchenne muscular dystrophy muscle is the rapid depletion of endogenous muscle progenitor cells. Therefore, using normal stem cells to promote muscle regeneration represents a potential therapeutic approach. It has been known that the differentiation of cells into a particular tissue type is regulated by a group of proteins with TGF-beta as prototype. This research aims to increase the effectiveness of stem-cell-based therapy by decreasing the intrinsic effecter molecules inside the cells that relay the signals activated by TGF-beta and a related molecule, myostatin, which are known to counteract the differentiation into muscle cells. We aim to achieve this goal by modulating these signals in both the donor stem cells and recipient dystrophic muscles.

Fedik Rahimov Ph.D.

DG Biomarker Discovery in Muscles from FSHD Patients

Summary In facioscapulohumeral muscular dystrophy (FSHD), overexpression of the DUX4 gene from the contracted D4Z4 locus is believed to induce variable degrees of myofiber degeneration and muscle weakness in different patients. Although the primary cause of FSHD is known, the underlying molecular mechanisms responsible for the progressive and selective degeneration of muscles are poorly understood. We will use mRNA expression profiling as an approach to understand these mechanisms with the hope that this understanding might lead to the discovery of biomarkers. mRNA-based biomarkers that we aim to identify in this study will be valuable for developing and assessing the success of possible therapies for this currently untreatable disease.

Boston - Dana-Farber Cancer Institute Pere Puigserver PhD

RG Role of mTOR/YY1/PGC1 transcriptional complex in mitochondrial myopathies. \$101,443.00 8/1/2012 7/31/2013 Year 2 \$101,443.00 8/1/2013 7/31/2014 Year 3

Summary Mitochondria are the energetic power plants of the cell that use nutrients as a fuel to obtain energy required to maintain cellular functions and survival. Failure to activate normal mitochondrial function is a key feature of mitochondrial myopathies, a group of neuromuscular disorders, often caused by mutations affecting proteins from this organelle. There is currently no therapy to treat mitochondrial myopathies which present a progressively severe neuromuscular dysfunction. The transcriptional metabolic coactivator PGC-1a rescues some of the defective mitochondrial function both in cell culture and mouse models of mitochondrial myopathies. Importantly, activation of PGC-1a function prevented a energetic deficit and effectively improve the mitochondrial myopathy phenotype in mouse models. Thus, activation of PGC-1a, or components of its molecular pathway, could be a treatment for mitochondrial myopathy patients. However, because PGC-1a is a transcriptional coactivator small molecules that bind directly and activate its function are unlikely to be found. Based on our knowledge of how the PGC-1a pathway is activated, here we propose two new strategies. First, activation of PGC-1a through the mTOR/YY1 complex and, second, small molecule chemical screening to identify compounds that activate PGC-1a using cybrid cell lines carrying genetic mutations from mitochondrial myopathy patients.

Boston - Harvard Medical School Alfred L. Goldberg Ph.D.

RG Protein breakdown in muscle in normal and disease states

Our studies during the past grant period have further clarified the common mechanisms of muscle wasting in a variety of pathological conditions including motor neuron disease (e.g. ALS), various myopathies, and systemic diseases. We showed that the ubiquitin-proteasome pathway is critical in destroying the myofibril during denervation atrophy. We made the unexpected finding that different components of the contractile apparatus are lost in a distinct order and that the enzyme, MuRF1, which is dramatically induced during all known types of atrophy, targets components of the muscle's thick filaments for destruction by the proteasome. However, the components of the thin filament are targeted by distinct enzymes. Previously, we demonstrated that the transcription factor, FoxO3, is critical in various types of muscle atrophy by causing expression of a set of atrophy-related genes (e.g. MuRF1), and that activation of FoxO3 alone causes profound muscle wasting. We also discovered an important new role of FoxO in stimulating autophagy, which catalyzes the destruction of mitochondria during atrophy. FoxO3 increases expression of many components of the autophagy process, which we showed are also induced in mouse muscles atrophying in vivo. Finally, we have shown

that exercise inhibits atrophy in part by inducing production of PGC-1alpha, which blocks the ability of FoxO to stimulate protein breakdown by these mechanisms.

Edward Owusu-Ansah Ph.D

DG	A Molecular Genetic Analysis of Mitochondrial Myopathy				
	\$60,000.00	8/1/2012	7/31/2013	Year 2	
	\$60,000.00	8/1/2013	7/31/2014	Year 3	

Summary A functional oxidative phosphorylation (OXPHOS) system is crucial for the generation of ATP (energy) in mitochondria. Accordingly, disruption of this system can compromise a range of biochemical and metabolic activities in cells, resulting in several muscle and neurodegenerative diseases. Mitochondrial Complex I deficiency is associated with many mitochondrial diseases, yet the molecular pathways that are disrupted upon complex I disruption, which trigger downstream signaling events associated with disease, remain largely unresolved. Damaged mitochondria compensate by increasing the expression of genes that allow survival under suboptimal conditions. Most of these damage-induced genes are involved in either repairing the damaged mitochondria or making new mitochondria. The full complement of cellular processes activated to restore or maintain viability in the presence of damaged mitochondria constitute an adaptive cytoprotective response. We have established a paradigm in the model organism Drosophila to study cytoprotective factors activated in response to mitochondrial perturbation. Due to the extensive similarity between Drosophila and human genomes, we anticipate that information obtained from this study should uncover novel therapeutic strategies for alleviating mitochondrial diseases in humans, and ultimately the aging process.

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	8/1/2012	7/31/2013	Year 1		
	\$100,538.00	8/1/2013	7/31/2014	Year 2		
	\$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.) James D. Berry M.D., M.P.H.

CRTG	A Phase I/II Study o	f a Continuous Infusion of	ISIS 333611 in SOD1 F	amilial ALS
	\$90.000.00	7/1/2012	6/30/2013	Year 2

Summary Amyotrophic lateral sclerosis (ALS) is a devastating neurologic illness causing progressive weakness. 10-20% of ALS is familial, or inherited, and often progresses to death within a year. Of patients with familial ALS, 20% have a mutation in a gene called superoxide dismutase, or SOD1. This gene creates abnormal RNA, which in turn creates abnormal SOD1 protein, causing the disease. The study medication, ISIS 333611, binds to SOD1 RNA and signals it for destruction. We think that this will

slow ALS progression. The medication must be given into the spinal canal (intrathecal space) by a pump implanted beneath the skin. ISIS 333611 was safe and effective in animal testing, and a preliminary human study is ongoing. In the proposed trial, we will give the medication to 24 familial ALS patients for 28 days and assess safety. We will test 3 dosages in groups of 8 patients. Two patients in each group of 8 will receive placebo instead of study medication. This helps determine if an effect, or side effect, is due to the medication. We will also track levels of the medication in the blood and spinal fluid, measure levels of SOD1 in the spinal fluid, and track patients' disease severity with forced vital capacity and the revised ALS Functional Rating Scale. Because this is an early trial, we do not expect to prove the drug is effective, only that it is safe and well tolerated. If so, a larger study will be conducted to evaluate its effectiveness.

Andrew Brack PhD

RG	Maintenance of the satellite cell pool in murine dystrophic muscle				
	\$117,701.00	2/1/2012	1/31/2013	Year 2	
	\$117,701.00	2/1/2013	1/31/2014	Year 3	

Summary Muscle satellite cells are the major the source of cells called into action to repair damaged muscle. They are normally in a functionally dormant state called quiescence, which prevents their depletion throughout life. However in muscular dystrophy, the continuous degeneration/regeneration cycles of the muscle puts extra demands on the satellite cell pool, resulting in their precocious expansion and ultimately their exhaustion. By understanding how satellite cells retain their quiescent state we can begin to unravel the mechanisms controlling the size of the satellite cell pool. In Sprouty1, we believe we have found both a marker and regulator of quiescent satellite cells and thereby offer a potential mechanism to enhance muscle repair throughout life.

Merit Ester Cudkowicz MD

RG	Trial of high fat/high calorie diet versus optimal calorie replacement in ALS				
	\$.00	7/1/2011	6/30/2013	Year 3	

Summary Weight loss is a common and severe symptom of amyotrophic lateral sclerosis (ALS), caused both from inadequate calorie intake and an increased metabolic rate. People with ALS are generally instructed to increase their calorie intake; however, the ideal amount and type of calories has not been studied. Several studies in an animal model of motor neuron disease have shown that a high fat/high calorie diet can increase survival by as much as 38%. Mice on a high fat diet also live longer than mice fed diets consisting of high protein or high sugar. This is a phase II safety, tolerability, and preliminary efficacy trial in ALS of high fat versus high calorie versus normal diet through the MDA ALS Clinical Research Network. Each intervention diet will be calculated based on the optimal calorie requirements needed to replace participants' measured energy expenditure. Aim 1 is to measure feasibility, compliance and serious adverse events on the three diets. Aim 2 is to measure markers of lipid metabolism and body composition before and after the study interventions. Aim 3 is to look at preliminary outcome measures including weight, BMI, the ALS Functional Rating Scale –Revised, forced vital capacity, grip strength and survival.

Vera Fridman M.D.

CRTG	Effect of L-serine supplementation on clinical progression in HSAN1					
	\$90,000.00	9/16/2013	9/15/2014	Year 1		
	\$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.

Boston - Trustees of Boston University

Mahasweta Girgenrath Ph.D

RG	Modulation of Inflammation in the context of Regeneration in MDC1A				
	\$119,183.00	2/1/2012	1/31/2013	Year 1	
	\$119,133.00	2/1/2013	1/31/2014	Year 2	
	\$119.149.00	2/1/2014	1/31/2015	Year 3	

Summary Congenital muscular dystrophies (CMD) exist in many different forms, the second most prevalent being type 1A (MDC1A). Children with MDC1A have poor muscle tone at birth, extremely compromised neuromuscular function and are rarely able to achieve independent ambulatory capacity. In many cases these children succumb to premature death either due to respiratory complications or failure to thrive. At present, there is no effective therapy in place to treat this lethal form of muscular dystrophy. This project proposes to explore combinatorial strategies (targeting inflammation and/or fibrosis along side of regeneration) to improve pathologies associate with muscular dystrophy resulting due to lack of laminin alpha 2, an extracellular matrix protein.

Jeffrey Boone Miller PhD

DC '	CNAD Q I CNAD the sure			11-	
RG	CMD & LGMD therapeutic targets: Studies with patients' myogenic cells				
	\$114,620.00	2/1/2012	1/31/2013	Year 1	
	\$114,620.00	2/1/2013	1/31/2014	Year 2	
	\$114,620.00	2/1/2014	1/31/2015	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.

Cambridge - Acceleron Pharma

chris rovaldi M.Sc.

MVP Phase IIa trial of ACE-031 for the treatment of Duchenne Muscular Dystrophy \$.00 7/1/2012 1/15/2013 Year 3

Summary ActRIIB is the high affinity receptor for GDF-8 (myostatin), a well-studied negative regulator of muscle mass and strength. ACE-031 binds tightly to GDF-8 and other negative regulators of muscle mass thereby inhibiting their biological effects. ACE-031 treatment increases muscle mass and strength in preclinical models of muscle loss and weakness including models of Duchenne muscular dystrophy. The available non-clinical safety and efficacy data in conjunction with the safety tolerability, PK and PD data from the Phase 1 healthy volunteer studies supports the rationale to evaluate the safety, tolerability, PK and PD of ACE-031 in patients with Duchenne muscular dystrophy. This proposal is to support a Phase II trial in DMD patients.

Cambridge - ALS Therapy Development Foundation Inc.

Steven Perrin Ph.D.

MVP Augie's Quest Grant to ALSTDI 2013

\$3,200,000.00 1/1/2013 12/31/2013 Year 1

Summary

Concord - Valerion Therapeutics, Inc.

Dustin Armstrong Ph.D.

MVP	Muscle Targeted Myotubularin 1 for Treatment of Congenital Myotubular	1 for Treatment of Congenital Myotubular
	Myopathy	

\$140,612.00	4/30/2013	4/30/2014	Year 1
\$1.055.150.00	4/30/2014	4/30/2015	Year 2

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

Worcester - University of Massachusetts Medical School Charles P. Emerson Ph.D.

RG	Evaluating DUX4 as a therapeutic target for FSHD				
	\$200,000.00	2/1/2012	1/31/2013	Year 1	
	\$176,470.00	2/1/2013	1/31/2014	Year 2	
	\$176,470.00	2/1/2014	1/31/2015	Year 3	

Summary Facioscapulohumeral muscular dystrophy (FHSD) is linked to abnormalities on a section of chromosome 4 (4q35). These genetic and/or epigenetic abnormalities appear to lead to aberrant expression of a potentially toxic protein, named DUX4-fl, and this mis-expression of DUX4-fl is currently thought to underlie the development of muscle weakness in FSHD. However, many questions remain about how DUX4-fl mis-expression occurs in diseased muscle and how therapies might be designed to inhibit pathology caused by DUX4-fl. Our studies therefore are designed to answer important questions about how DUX4-fl expression is regulated. In particular, we will investigate the connection between changes in the DUX4 gene and expression of the toxic form of this gene to identify new therapeutic targets for FSHD. In addition, we will identify and validate potential targets for small molecule therapies. Furthermore, we will determine if drugs affecting the regulation of the DUX4 locus can eliminate expression of the toxic protein, raising their potential as FSHD therapeutics. We expect that the results of our studies will provide critical information needed to develop effective and specific DUX4-targeted therapies for FSHD.

Michael M. Francis PhD

RG	Genetic analysis of a C. elegans model of slow-channel myasthenic syndrome				
	\$110.000.00	7/1/2012	6/30/2013	Year 3	

Summary Alterations in the strength of cholinergic signaling at the neuromuscular junction (NMJ) underlie a variety of neurological disorders including congenital myasthenic syndromes and myasthenia gravis, and may contribute to the progressive muscle degeneration observed in Duchenne's muscular dystrophy. Therefore, gaining a detailed understanding of the molecular mechanisms that regulate NMJ development and function is of paramount importance for the development of strategies to combat these debilitating disorders. To gain new insights into the molecular pathways that guide the assembly and function of neuromuscular synapses, we have been studying the cholinergic NMJ of the highly tractable model organism Caenorhabditis elegans. Using the sophisticated genetic tools available in this system, we have developed a model of slow channel congenital myasthenic syndrome (SCCMS), enabling a comprehensive forward genetic analysis of the molecular pathways affected by this disorder. Our studies offer a unique opportunity to dissect evolutionarily conserved mechanisms that control NMJ development and maintenance, and will lead to a better understanding of the synaptic defects underlying NMJ disorders, particularly SCCMS. Further, we expect that our work will enable detailed studies of the genetic pathways involved in the neuromuscular degeneration observed in SCCMS, and contribute to the development of effective therapeutic interventions.

Rossella Tupler M.D., Ph.D.

RG Dissecting the complexity of FSHD molecular pathogenesis

\$130,000.00	8/1/2012	7/31/2013	Year 1
\$130,000.00	8/1/2013	7/31/2014	Year 2
\$.00	8/1/2014	7/31/2015	Year 3

Facioscapulohumeral muscular dystrophy (FSHD) has been associated with a reduction Summary in the number (= 8) of D4Z4 elements at 4g combined with a specific set of DNA markers (4A159/161/168-PAS haplotype), which provides the possibility of expressing DUX4 gene. However, families studied in Italy, Brazil and the United States suggest that D4Z4 reduction and DUX4 expression are not sufficient to cause disease. In Italian families, study of 253 unrelated FSHD patients revealed that 204 (80.6%) carry D4Z4 alleles with 1-8 units, 19 (7.5%) have D4Z4 alleles with 9-10 repeats, and 30 (11.8%) carry D4Z4 alleles with 11 repeats or more on both chromosomes 4. Analysis of the 4q35 haplotype of these 253 unrelated FSHD patients showed that only 127 of them (50.1%) carry the 4A159/161/168-PAS haplotype. In the United States, family members with identical D4Z4 repeat lengths included members who were non-manifesting and members with classical FSHD and not all families express DUX4. Finally, analysis of the 4q haplotype of 801 healthy subjects from Italy and Brazil revealed that 1.3% of individuals carry the 4A161PAS haplotype. Collectively these results indicate that FSHD etiology is more complex than expected. With this research we will sequence all the coding genes of the whole genome (exome sequencing) in a selected group of FSHD patients and relatives to identify genetic elements that contribute to the disease onset.

MICHIGAN

Ann Arbor - Nymirum, Inc.

Michael Pape PhD

RG SAR for Small Molecules Targeting Toxic CUG Expanded Repeats.

\$197,066.00 8/1/2013 7/31/2014 Year 1

Myotonic dystrophy type 1 (DM1) is caused by expansion of CTG repeats located in the Summary dystrophia myotonia protein kinase (DMPK) gene. Transcription of expanded CTG repeats to expanded CUG repeats (CUGexp) causes the DMPK pre-mRNA to b-e trapped in the nucleus preventing export to the cytoplasm resulting in decreased cellular levels of DMPK protein. Interestingly, low levels of DMPK protein are not the major cause of DM1, but rather expression of CUGexp appears to cause aberrations in splicing integrity. Of particular interest is the pre-mRNA splicing regulator protein muscleblind-like 1 (MBNL1), which binds the CUGexp RNA hairpin structure and becomes trapped in nuclear foci. In total, the splicing derangements result in skeletal muscle hyperexcitability causing myotonia. Several studies using small molecules and oligonucleotides show that CUG-binding ligands can inhibit MBNL1 binding to CUGexp and alleviate CUGexp mediated dysfunctions in vivo. While these studies provide proof of concept, none of the small molecules possess the required efficacy and/or drug-like properties (e.g. oral bioavailability and safety window) for chronic treatment. Nymirum has discovered two drug-like chemical series that bind CUG, alleviate CUGexp mis-splicing in cell-based assays, and exhibit robust SAR. Here, we will investigate rational chemical modifications to increase their affinity to CUGexp and enhance their potency in cell-based mis-splicing assays.

Ann Arbor - The Regents of the University of Michigan Eva L. Feldman M.D., Ph.D.

RG	Neuromuscular junction transmission and myotubular myopathy				
	\$117,893.00	2/1/2012	1/31/2013	Year 2	
	\$117.893.00	2/1/2013	1/31/2014	Year 3	

Summary Myotubular myopathy (MTM) is caused by mutations in the myotubularin gene. The function of myotubularin is known, but the understanding of why this function is important for muscle and why loss of its function causes severe muscle disease is only now coming into focus. Currently there is no treatment for MTM; thus, our goal is to identify new potential therapies. Recently we identified abnormalities in a part of the muscle called the neuromuscular junction (NMJ) in a model of the disease. We also found that treatment targeted to the NMJ greatly improved function in this model. Based on

these results, we hypothesize that NMJ abnormalities are an essential aspect of MTM and that treatment with drugs that modify the NMJ will improve the course of the disease. The goal of this proposal is to test these hypotheses. To do this, we will use a mouse model of MTM and (a) comprehensively dissect the relationship between the NMJ and the disease and (b) determine the effect on disease progression of a commonly used NMJ-modifying drug. Data gathered from these studies will significantly advance our understanding of myotubular myopathy and has high potential to directly lead to treatment for patients with this devastating disease.

Andrew Lieberman M.D., Ph.D.

RG	Allosteric activators of Hsp70 to treat spinobulbar muscular atrophy				
	\$34,375.00	8/1/2012	7/31/2013	Year 1	
	\$34,375.00	8/1/2013	7/31/2014	Year 2	
	\$34,375.00	8/1/2014	7/31/2015	Year 3	

Spinobulbar muscular atrophy (SBMA) is an inherited degenerative disorder of lower *Summary* 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	8/1/2012	7/31/2013	Year 1	
	\$121,655.00	8/1/2013	7/31/2014	Year 2	
	\$121,655.00	8/1/2014	7/31/2015	Year 3	

Muscular dystrophies are characterized by muscles that are weak, sensitive to injury, Summary 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.

East Lansing - Michigan State University William David Atchison Ph.D.

RG

Compensatory mechanisms in acetylcholine release in Lambert-Eaton Syndrome

\$102,909.00	2/1/2012	1/31/2013	Year 2
\$102,909.00	2/1/2013	1/31/2014	Year 3

Summary Lambert-Eaton Syndrome (LEMS) is a disorder of neuromuscular transmission which occurs in patients with certain forms of lung cancer. LEMS results from impaired release of the chemical messenger, acetylcholine, which controls muscle function. Acetylcholine release from nerves during muscle activity is controlled by proteins in the nerve ending called calcium channels. In LEMS, the type of calcium channel which controls release of the messenger acetylcholine is attacked by antibodies. Thus muscle weakness occurs. We found that during LEMS, different types of calcium channels try to compensate for the loss of the normal calcium channel protein (called P/Q-type). We are studying how this compensatory process occurs, and whether it could be used as a target for therapy for patients with LEMS. To do this, we treat mice with plasma from patients with LEMS, and measure how the compensatory calcium channel begins to contribute to neuromuscular function LEMS. We are using mice with genetic mutations in the calcium channel to find out if this is the only target in LEMs, or whether other proteins are also affected. We also want to know if the compensatory channels can relocate to the site at which the normal acetylcholine release occurs.

MINNESOTA

Minneapolis - Regents of the University of Minnesota - Twin Cities Atsushi Asakura Ph.D.

RG	Angiogenesis-based therapy for muscular dystrophy				
	\$134,851.00	8/1/2012	7/31/2013	Year 1	
	\$121,840.00	8/1/2013	7/31/2014	Year 2	
	\$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.

James M. Ervasti Ph.D.

RG	Biophysical Optimization of Therapeutic Dystrophin Constructs				
	\$130,000.00	2/1/2012	1/31/2013	Year 1	
	\$130,000.00	2/1/2013	1/31/2014	Year 2	
	\$130,000.00	2/1/2014	1/31/2015	Year 3	

Summary Although many therapeutic approaches for Duchenne muscular dystrophy have shown promise, no cure for patients is currently available. Several strategies are directed toward restoring dystrophin expression, or replacing its function through exon skipping, upregulation of the dystrophin homolog utrophin, and viral delivery of miniaturized dystrophin or utrophin gene constructs. The use of miniaturized dystrophin/utrophin constructs and skipping approaches each rely on the presumed functionality of proteins bearing non-native junctions. Over the past 17 years, we have elucidated many features of full-length dystrophin and utrophin structure/function. With regard to the above-mentioned therapeutic approaches, new studies suggest that the functionality of internally truncated dystrophin constructs appear to be compromised by protein instability and aggregation. Therefore, we will apply our unique knowledge and technical capabilities to perform a complete characterization of internally truncated, therapeutically-relevant dystrophin proteins with the ultimate goal of maximizing efficacy via optimization of protein stability.

Michael Kyba PhD

RG Development of anti-DUX4/D4Z4 therapeutics and testing in animal models

\$125,000.00	8/1/2012	7/31/2013	Year 2
\$125,000.00	8/1/2013	7/31/2014	Year 3

Summary The DNA lesion associated with this facioscapulohumeral muscular dystrophy (FSHD)is a contraction within a series of 3.3 kb repeats (D4Z4 repeats) near the telomere of 4q. It is not understood how this contraction results in disease, however it appears to modify the chromatin configuration of 4q35.2 and this has been proposed to lead to derepression of the locus, resulting in misexpression of a gene named DUX4 encoded within each D4Z4 repeat. We are developing pharmacological and genetic inhibitors of DUX4 as well as mice that carry the DUX4 gene. We propose to identify promising inhibitors of DUX4 as lead candidate drugs to treat FSHD, and to test these in our DUX4-transgenic mice.

Michael Kyba PhD

RG	FSHD iPS cells: genetic correction and myogenesis				
	\$119,409.00	2/1/2013	1/31/2014	Year 1	
	\$119,409.00	2/1/2014	1/31/2015	Year 2	
	\$119.409.00	2/1/2015	1/31/2016	Year 3	

Summary The mechanism of muscle degeneration in FSHD is engimatic. Unlike Duchenne's and other Dystroglycanopathies, FSHD neither presents evidence of ongoing muscle fiber damage, nor evidence of ongoing regeneration. The mutation that causes FSHD is known, but the way in which that mutation affects muscle physiology is not understood. We have generated iPS cells from FSHD patients. These are cells from the skin that have been reprogrammed into an earlier state, such that they can differentiate into any cell type in the petri dish. We propose to use newly-developed gene targeting technology to correct the FSHD mutation, and to study muscle development from the parental and the corrected iPS cells.

Joseph M Metzger Ph.D

RG	Development and testing of membrane sealants for muscular dystrophy				
	\$118,934.00	8/1/2012	7/31/2013	Year 1	
	\$118,934.00	8/1/2013	7/31/2014	Year 2	
	\$.00	8/1/2014	7/31/2015	Year 3	

Summary Dystrophic skeletal and cardiac muscle have weakened cellular membranes that render the muscle tissue highly susceptible to contraction-based injury and ultimate destruction. Our strategy is to forge discovery of membrane protective molecules for clinical application. This project's rational design of molecular band aids (copolymer molecules) for muscle membrane protection has the potential to spark a revolution in novel therapeutics for diseased striated muscles in muscle dystrophy.

Rita R. Perlingeiro Ph.D.

RG	DMD IPS CELLS: GENETIC CORRECTION AND MUSCLE REGENERATION					
	\$130,000.00	8/1/2012	7/31/2013	Year 1		
	\$130,000.00	8/1/2013	7/31/2014	Year 2		
	\$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.

David D. Thomas Ph.D.

RG	Muscular dystrophy therapy based on small-molecule activators of Ca2+ transport					
	\$130,000.00	2/1/2012	1/31/2013	Year 1		
	\$130,000.00	2/1/2013	1/31/2014	Year 2		
	\$130,000.00	2/1/2014	1/31/2015	Year 3		

Summary Increased calcium influx, and reduced calcium removal from the sarcoplasm are thought to contribute to the muscular dystrophy (MD) phenotype. This suggests that activating Ca-transport (pumping) out of the sarcoplasm should benefit MD individuals. To test this hypothesis, we will use small-molecule compounds that specifically activate the calcium pump (SERCA) from the sarcoplasmic reticulum (SR), the intracellular reservoir of calcium. Our goal is to test whether compounds that activate SERCA in vitro can improve muscle contractility in vivo (and reduce MD pathology), in mouse MD models. The results of this research will provide proof-of-concept for a small-molecule strategy to treat MD and help select a set of lead compounds for drug development.

Rochester - Mayo Clinic.

Andrew George Engel M.D.

RRG Restricted funds for congenital myasthenia gravis research

\$200.00 1/1/2013 12/31/2013 Year 2

Summary

MISSOURI

Columbia - The Curators of the University of Missouri

Dawn DW Cornelison Ph.D.

RG Functional domains of syndecan-4 mediate distinct satellite cell activities

\$.00 7/1/2012 6/30/2013 Year 3

Summary Upon muscle injury, satellite cells (the stem cells of skeletal muscle) respond to a dynamic suite of extracellular signals to sequentially activate, proliferate, migrate, and differentiate into new muscle fibers. In diseases such as Duchenne Muscular Dystrophy, the capacity of satellite cells to respond to the ongoing muscle damage eventually becomes depleted, for reasons that are not yet well understood. The potential for cell-based replacement therapies, in which satellite cells carrying a 'good' copy of the dystrophin gene are transplanted into DMD muscle, has advanced significantly since the identification of protein 'markers' that confer an enhanced capacity to replace the patient's satellite cells. However, predicting and controlling the behavior of cells after they are injected has proved problematic. One satellite cell protein, syndecan-4, is both a marker of cells that engraft as new satellite cells and a mediator of all four processes mentioned above. We show that different regions of the syndecan-4 protein are required to promote growth or differentiation; both of these regions act by binding to other intracellular proteins. We will determine the identity of the proteins that bind to each specific region, and then ask how each interaction leads to signals that enhance either cell division or muscle differentiation.

Dongsheng Duan PhD

RG Improving AAV potency for DMD gene therapy

\$175,890.00 2/1/2012 1/31/2013 Year 2 \$175,890.00 2/1/2013 1/31/2014 Year 3

Summary Duchenne muscular dystrophy (DMD) is a fatal disease caused by dystrophin gene mutation. Adeno-associated virus (AAV)-mediated gene therapy has shown great promise to ameliorate this disease. Recent development in systemic AAV delivery further raises the hope of whole-body correction. However, systemic AAV delivery requires trillions of AAV particles. This creates a tremendous workload for vector production and it may also stimulate untoward side effects. We recently found that a novel AAV-9.7 vector was 10-fold more potent than the current AAV vectors in mice. We have also developed a novel nNOS recruiting micro-dystrophin gene that is functionally superior to the ones currently in use. In this study we will combine the new AAV vector and the new microgene to DMD gene therapy in the mouse and dog models of DMD. Our study will accelerate the tempo of DMD gene therapy research and facilitates its translation to human patients in the future.

Christian Lorson PhD

RG Evaluation of SMA pathways with scAAV9 vectors

\$127,194.00 2/1/2012 1/31/2013 Year 1

\$127,194.00	2/1/2013	1/31/2014	Year 2
\$127,194.00	2/1/2014	1/31/2015	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 - Washington University in St.Louis Bogdan Karl Beirowski M.D., Ph.D.

DG	Modeling axon loss in CMT by disruption of Schwann cell metabolic regulation				
	\$60,000.00	8/1/2012	7/31/2013	Year 1	
	\$60,000.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Martha Bhattacharya Ph.D.

DG	Molecular Mechanisms of Peripheral Axonal Degeneration			
	\$60,000.00	2/1/2013	1/31/2014	Year 1
	\$60,000.00	2/1/2014	1/31/2015	Year 2
	\$60,000.00	2/1/2015	1/31/2016	Year 3

In neuromuscular diseases where motoneuron dysfunction is the primary cause of Summary 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.

Anne M Connolly M.D.

RG	Clinical Outcome validation	lation in Non-ambulatory and young Boys/Men w		n with DMD
	\$203.080.00	1/1/2012	9/30/2013	Year 3

Summary In this project the five named DMD-MDA centers will establish clinical outcome measures in young boys and wheelchair-bound boys and men with DMD with the plan that they may be used in future clinical trials. There is a pressing need now to establish outcomes for very young who may be unreliable for testing and for older, weaker boys and men. Currently only ambulatory boys qualify for most clinical studies. Thus, more that 75% of boys and men with DMD are not eligible. We will begin this work in older boys and men with DMD in year one of this grant and establish reliability in measurements of 1) arm and hand function as well as strength, 2) contractures 3) vital capacity (lung function), and quality of life. We will next study and assess reliability of the measure of gross motor and intellectual development in young boys with DMD. Finally we will use the outcomes as part of the proposed study of ace inhibition versus angiotensin receptor blockade in conjunction with a proposed study by Dr. Jerry Mendell and colleagues.

Anne M Connolly M.D.

HCT	Phase 2 Historically Controlled Trial of Corticosteroids in Young Boys with DMD			
	\$49,794.00	8/1/2013	12/31/2013	Year 1
	\$157,133.00	1/1/2014	12/31/2014	Year 2
	\$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	8/1/2012	7/31/2013	Year 1	
	\$119,122.00	8/1/2013	7/31/2014	Year 2	
	\$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

HCT	Natural History Study of Familial ALS				
	\$113,663.00	1/1/2013	12/31/2013	Year 2	
	\$.00	1/1/2014	6/30/2014	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.

Conrad Weihl M.D., Ph.D.

RG	Autophagic treatment paradigms for desminopathies				
	\$132,916.00	2/1/2012	1/31/2013	Year 1	
	\$132,074.00	2/1/2013	1/31/2014	Year 2	
	\$132,074.00	2/1/2014	1/31/2015	Year 3	

Summary Protein aggregate myopathies are a group of devastating muscular disorders with common pathologic features. These include focal disruption of myofibrils, the accumulation of undegraded myofibrillar proteins and the ectopic aggregation of multiple proteins including desmin, amyloid precursor protein (APP), TAR DNA-binding protein 43 (TDP-43), áB-crystallin, and ubiquitin. Currently no treatment exists for these disorders. In order to study the pathogenesis and evaluate potential therapeutics for protein aggregate disorders, we generated an áB-crystallin knockin mouse model. This mouse expresses mutant R120G áB-crystallin under its endogenous promoter making it an ideal model for this multisystem disease. Both heterozygous and homozygous knockin mice are viable and develop skeletal muscle weakness, protein inclusions and cataracts. In skeletal muscle, these inclusions are insoluble and contain áB-crystallin, desmin and ubiquitin. We have developed assays to measure the autophagy in skeletal muscle. We will use these assays to define FDA approved drugs with reported autophagic activity. These drugs will then be used to treat an animal model of desmin related myopathy. Upon completion of these studies we will know if autophagic enhancement is a viable therapeutic approach in this devastating disorder.

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	8/1/2012	7/31/2013	Year 1	
	\$102,676.00	8/1/2013	7/31/2014	Year 2	
	\$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 frag	ment and peptide therap	y for Duchenne Muscu	ılar Dystrophy
	\$60,000.00	8/1/2012	7/31/2013	Year 1
	\$60,000.00	8/1/2013	7/31/2014	Year 2
	\$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 N/A

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

\$60,000.00	6/1/2013	5/31/2014	Year 1
\$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.

Newark - Rutgers, The State University -Newark Campus Diego Fraidenraich Ph.D.

RG	Pluripotent stem cell-induced corrections in muscle and fat of mdx mice				
	\$125,000.00	8/1/2012	7/31/2013	Year 2	
	\$125,000,00	8/1/2013	7/31/2014	Year 3	

Summary Interactions between adipose and muscle have attracted the attention of the scientific and medical communities lately. Recent published reports demonstrate that skeletal muscle and one type of fat have a common cellular ancestor, and our studies of developing mice show related changes in these tissues. We treated an embryonic mouse model of Duchenne muscular dystrophy with stem cells derived from normal mouse tissue in order to provide the missing muscle protein. New, normalized muscle developed, but there was also an increase in fat and a persistence of muscle markers in fat tissue that are not seen in normal mice. In this project we will characterize this stem cell-derived fat and understand its role in the development of normal muscle. New determinants of muscle formation will provide mechanisms for future therapies. Key words: fat/muscle conversion, blastocyst injection, embryonic and induced pluripotent stem cells, mdx

NEW MEXICO

Albuquerque - The Regents of the University of New Mexico Richard Cripps D. Phil.

RG A Drosophila model for mammalian muscular dystrophy \$113,187.00 7/1/2012 6/30/2013 Year 3

Summary We shall study muscle development in the fruit fly, Drosophila melanogaster, to help us understand muscle development and disease in humans. We use Drosophila as a model organism because the mechanisms of muscle development in this animal are very similar to those of vertebrates, yet the genetic processes are simpler and more well understood in flies. This project will study the function in Drosophila of a gene named abba, for which mutations in a related gene in humans have been identified to cause muscular dystrophy. We shall carry out experiments to understand how abba works in flies, including characterizing mutants for this gene, which we propose will develop muscular

dystrophy in fly larvae. We shall also carry out experiments to define at the molecular level how the protein produced by abba functions in muscle, which will tell us a great deal about how the normal gene works in humans. These findings in Drosophila will therefore provide insight into normal muscle processes in humans; accordingly, our data will help us to understand how muscle development goes awry in diseased individuals, and will uncover potential mechanisms by which to generate rational therapies for muscle disease.

Albuquerque - University of New Mexico HSC Sarah Youssof M.D.

CRTG	Outcome Measures in Oculopharyngeal Muscular Dystrophy				
	\$90,000.00	7/1/2013	6/30/2014	Year 1	
	\$90.000.00	7/1/2014	6/30/2015	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	2/1/2013	1/31/2014	Year 1
	\$135,000.00	2/1/2014	1/31/2015	Year 2
	\$135,000.00	2/1/2015	1/31/2016	Year 3

Excessive activation of the a-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) Summary 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.

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				
	\$141,415.00	2/1/2012	1/31/2013	Year 1	
	\$138,608.00	2/1/2013	1/31/2014	Year 2	
	\$134,764.00	2/1/2014	1/31/2015	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.

Cold Spring Harbor - Cold Spring Harbor Laboratory Kentaro Sahashi M.D., Ph.D.

DG	Phenocopying SMA in mice using antisense oligonucleotides				
	\$60,000.00	2/1/2012	1/31/2013	Year 1	
	\$60,000.00	2/1/2013	1/31/2014	Year 2	
	\$60,000.00	2/1/2014	1/31/2015	Year 3	

SMA is caused by mutations in the SMN1 gene. A closely related SMN2 gene expresses Summary low levels of functional SMN protein, due to incorrect splicing. Splicing is an RNA cutting and pasting step required for translating the coding segments of genes (exons) into a functional protein. Antisense oligonucleotides (ASOs) are versatile molecules that can be used to alter splicing patterns of target RNAs. Currently available SMA mouse models, while extremely useful, fall short of providing accurate models for intermediate forms of SMA that would allow detailed analyses of molecular, physiological, and phenotypic features of SMA. We recently designed a pathogenic ASO that exacerbates SMN2 missplicing, inducing more severe SMA in transgenic mice harboring human SMN2. Preliminary ASO injection into the central nervous system of neonatal mice recapitulated SMA-like progressive motor dysfunction, growth impairment, and shortened life span. The ASO treatment promoted SMN2 missplicing in the spinal cord at postnatal day 7 (P7). We observed further inhibition at P30, indicative of a delayed splicing effect and/or the consequence of SMA progression. Pathogenic ASOs that cause sustained splicing defects can potentially be used as a general strategy to model certain diseases in animals. By exploring the effects of ASO's in different tissues, and at different developmental stages, I will obtain relevant insights into the roles of SMN in SMA pathogenesis, as well as its normal functions.

Ithaca - Cornell University Fenghua Hu Ph.D.

RG	Role of Ubiquitination in TDP-43 aggregation and clearance				
	\$120,000.00	2/1/2013	1/31/2014	Year 1	
	\$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 College of Physicians and Surgeons Howard J. Worman M.D.

RG Treatment of Cardiomyopathy in Emery-Dreifuss Muscular Dystrophy \$103,631.00 7/1/2012 6/30/2013 Year 3

Summary The life-threatening complication of Emery-Dreifuss muscular dystrophy is a disorder of the heart muscle known as cardiomyopathy. When this is advanced, the only currently available curative treatment is heart transplantation. We have shown in a mouse model of Emery-Dreifuss muscular dystrophy that treatment with drugs that inhibit enzymes known as MAP kinases prevent the development of cardiomyopathy and improves heart function after deterioration has already begun. Similar drugs have already been given to humans for other indications. We now propose to expand our preclinical studies on MAP kinase inhibitors to treat cardiomypathy in Emery-Dreifuss muscular dystrophy with the ultimate goal of developing treatments for human patients with the disease. In this new project, we will examine novel MAP kinase inhibitors in additional mouse models of Emery-Dreifuss muscular dystrophy.

New York - Columbia University Medical Center Tomoyuki Awano Ph.D

DG	Investigating the existence and role of genetic modifiers of SMA in model mice				
	\$60,000.00	2/1/2012	1/31/2013	Year 1	
	\$60,000.00	2/1/2013	1/31/2014	Year 2	
	\$60,000.00	2/1/2014	1/31/2015	Year 3	

Summarv Spinal muscular atrophy (SMA) is a debilitating pediatric motor neuron disorder caused by SMN1 gene deletions. However, all patients bear one or more copies of an almost identical but partially functional copy gene, SMN2. Disease severity generally correlates with copy number of SMN2. Although the cause of the disease is clear, the precise biochemical pathway(s) that link SMN depletion to neurodegeneration remains unclear. Reports in the literature indicate that in rare instances siblings with identical SMN2 copy number nonetheless display varying disease symptoms. The differences have been ascribed to genetic modifiers. We have generated mouse models of SMA. We find that the severity of the disease in the mice varies depending on the genetic strain utilized. This is analogous to the "discordant" siblings observed in the human population. In this application, we will identify genes that modify the SMA phenotype. First, we will precisely establish whether differences in genetic background do indeed affect disease severity in mutant mice and the extent of the modification. Once we have established the differences, we will use the power of mouse genetics and the concept of linkage disequilibrium (LD) to identify genes responsible for the phenotypic differences. Our results will begin not only to define disease relevant mechanisms underlying SMA pathology but also identify novel molecular targets that may be amenable to manipulation in future therapeutic strategies

Veronica Hinton Ph.D.

RG	Executive Functions in Boys with Dystrophinopathy				
	\$132,532.00	8/1/2012	7/31/2013	Year 1	
	\$132,532.00	8/1/2013	7/31/2014	Year 2	
	\$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	8/1/2012	7/31/2013	Year 1	
	\$132,844.00	8/1/2013	7/31/2014	Year 2	
	\$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	2/1/2013	1/31/2014	Year 1	
	\$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	2011-2012 Wings Four Independent Proposals				
	\$167,542.00	9/1/2011	8/31/2013	Year 1	

Summary We propose 4 independent projects. Project 1 aims to investigate whether or not oxidative stress is increased in ALS patients with impending respiratory failure. With successful

treatment with non-invasive ventilators (NIV), oxidative stress may be significantly reduced along with an increased quality of life. For project 2, we will study metabolic markers, mitochondrial dysfunction, and oxidative stress in fibroblasts from ALS patients, in collaboration with Dr. Giovanni Manfredi, Weill Cornell Medical School. We will attempt to discover if ells outside of the nervous system would provide a clue for the pathogeneses of the disease. In project 3, we will prepare a new study to prospectively look at the natural history of pure upper motor neuron dysfunction/disease (PUMND) and early PLS. Project 4 aims to explore the feasibility of studying epigenetic in ALS. We are collecting RNA, skin fibroblast and autopsy tissue to identify the best way to study epigenetic changes in ALS. We will prepare a future large project in epigenetic studies, which is novel but requires a fundamental exploration for the methodologies and effective objectives. The MDA Wings Over Wall Street Funds have been a driving force for our innovative and highly active research projects at the Eleanor and Lou Gehrig MDA/ALS Research Center.

Hiroshi Mitsumoto MD

RRG 2012 Wings Over Wall Street Research Projects Proposal

\$126,201.00 8/1/2012 7/31/2013 Year 1

Summary This MDA Wings Over Wall Street grant has been so essential for the research activities at the Eleanor and Lou Gehrig's MDA/ALS Research Center for more than 12 years. We have made numerous accomplishments through innovative research and patient care development. This year, we request support for a project to identify new genes responsible for the fast disease progression of ALS and genes responsible for the extremely slow disease progression of PLS. We also ask for support towards the development of the concept of the PLS Research Consortium and to continue ALS DNA Banking.

Hiroshi Mitsumoto MD

RRG 2013 Wings Over Wall Street Research Projects Proposal

\$121,376.00 9/1/2013 8/31/2014 Year 1

Summary The MDA Wings Over Wall Street Fund has been extremely supportive of the diverse research activities at the Eleanor and Lou Gehrig MDA/ALS Research Center. The title of Project 1 is "Strengthening the Statistical Capability of the ALS COSMOS Project." The title of Project 2 is, "Screening C9ORF72 in patients enrolled in the ALS COSMOS Study." Project 3 is somewhat different, because we would like to supplement the CDC grant. If the CDC grant is funded, its budget is limited and we need additional support to conduct the project as we proposed. We are requesting for the MDA Wings Fund to assume costs related to data management. However, if the CDC proposal should not be funded, we would like MDA Wings to consider Option 2, which is to support glutathione MRS and multimodal MRS. In summary, the MDA Wings Over Wall Street fund has become extremely valuable for our innovative research activities, as we seek to decipher underlying disease mechanisms to find a cure for this devastating disease.

Umrao R. Monani Ph.D.

RG	Elucidating the role of the SMN protein in the developing neuromuscular system				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Ji-Yeon Shin Ph.D

DG LAP1 involvement in the pathology of Emery-Dreifuss muscular dystrophy \$60,000.00 7/1/2012 6/30/2013 Year 3

Summary Although diagnosis for Emery-Dreifuss muscular dystrophy (EDMD)has been improved by the discovery of the most common genetic mutations that cause this disease, we still have a poor understanding of how these mutations cause muscular dystrophy. I have discovered that proteins encoded by the genes mutated in most cases of EDMD interact with another protein with an unknown function. I will study how this protein affects well-defined signaling pathways and muscle cell function in cultured cells and in mice. The research will lead to a better understanding of how specific genetic mutations cause EDMD. This will enable us to identify new processes, such as cell signaling pathways, that could be targets for the development of novel drugs to treat EDMD.

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	8/1/2013	7/31/2014	Year 1	
	\$100,000.00	8/1/2014	7/31/2015	Year 2	
	\$100,000,00	8/1/2015	7/31/2016	Year 3	

Mutations in mitochondrial DNA (mtDNA) result in respiratory chain (RC) defects and **Summary** 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	2/1/2013	1/31/2014	Year 1	
	\$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					
	\$137,331.00	8/1/2012	7/31/2013	Year 1		
	\$133,304.00	8/1/2013	7/31/2014	Year 2		
	\$128.634.00	8/1/2014	7/31/2015	Year 3		

Emery Dreifuss Muscular Dystrophy (EDMD) has been linked to mutations in LMNA, a **Summary** 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 microtubuleassociated 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.

Sonja Nowotschin Ph.D

DG	Muscle morphogenesis: investigating cellular behaviors and molecular mechanisms				
	\$54,546.00	8/1/2012	7/31/2013	Year 2	
	\$54.546.00	8/1/2013	7/31/2014	Year 3	

Summary Critical to the design of rational therapies for congenital human muscle diseases, including the many congenital muscular dystrophies (CMDs), is a rigorous understanding of their developmental origin. Genetic approaches in experimentally tractable model organisms are central to furthering our understanding of CMDs. The paraxial mesoderm is the cell population of the embryo that gives rise to the vertebral column and the axial skeletal muscles of the body, the tissues affected in CMDs. Disruption of paraxial mesoderm specification and development have severe consequences for the formation of the skeletal muscles of the body. This project seeks to combine live imaging with genetic approaches in mice to investigate the basic cellular behaviors and molecular mechanisms underlying paraxial mesoderm formation in mammals and to identify the stem cells giving rise to the paraxial mesoderm. Importantly, by investigating the molecular mechanisms operating in the embryo we will be able to formulate the molecular principles that may, in the future, assist in developing methods to reprogram adult or differentiated cells.

New York - The Hospital for Special Surgery Dale Lange M.D.

RG	Safety and Efficacy of SOD	1 Inhibition by Pyrimet	chamine in Familial ALS	
	\$95,000.00	4/1/2011	6/30/2013	Year 3

Summary ALS is sometimes caused by a mutation in a gene that produces an enzyme known as superoxide dismutase (SOD1). Interfering with production of this enzyme in mice with ALS causes significant slowing of progression. We have shown that some patients with familial ALS show a reduction in the level of SOD1 when taking the drug pyrimethamine. However, some patients have had problems with tolerating higher doses of the drug, which we believe is related to the rate and amount of increase in dose. We also found that the degree that SOD1 is lowered by pyrimethamine may vary with mutation. We will continue our studies with a different rate of increase in pyrimethamine dose and to expand our study sites so as to include as many different mutations as possible. This will enable us to see if there is indeed a differential effect which would give us insight into the mechanism by which this mutation produces disease and information about possible effect of therapy.

New York - The Trustees of Columbia University in the City of New York Salvatore DiMauro M.D.

RG	Therapeutic strategies in neutral lipid storage disease with myopathy				
	\$136,487.00	2/1/2012	1/31/2013	Year 1	
	\$136,487.00	2/1/2013	1/31/2014	Year 2	

Summary Neutral lipid storage disease with myopathy (NLSDM) is a new inborn error of lipid metabolism due to the deficiency of a cytoplasmic adipose triglyceride lipase (ATGL) that catalyzes the first step in the hydrolysis of neutral fats (triglycerides, TG) stored in lipid droplets. Mutations in the gene encoding ATGL (PNPLA2) have been associated with generalized lipid storage dominated clinically by a relatively late-onset myopathy and, sometimes, cardiomyopathy. We are following an 18-year-old woman with severe lipid storage myopathy and a retrotransposal insertion in PNPLA2. Although she has high serum creatine kinase (CK), she is still asymptomatic and an avid dancer. We also have secured fibroblasts from 4 additional patients with symptomatic NLSDM, three studied in collaboration with Italian colleagues and one recently diagnosed here at Columbia University Medical Center. By studying lipid-laden fibroblasts in vitro, we hope to identify therapeutic agents that might protect our young patient from inevitably developing progressive myopathy and alleviate the weakness in the already symptomatic patients. Specifically, we have confirmed recent results that long-acting beta agonists reverse lipid accumulation in NLSDM fibroblasts (Reilich et al, J Neurol 2011; May 5), and seek to further investigate this effect with a view toward a possible clinical trial.

Oliver Hobert Ph.D.

RG	Deciphering the function of	f the ALS gene Tdp-43 ।	using the C.elegans mo	del system
	\$124,837.00	7/1/2012	12/31/2013	Year 3

Summary In order to diagnose and treat ALS, it is important to understand the molecular events that underlie this disease. One gene known to cause ALS in human, called TDP-43, works in a manner that is not understood. Our goal is to better understand the function of this gene. To this end, we propose to study this gene in a simple invertebrate species, C.elegans, which offers the opportunity to identify other genes that interact with this human disease gene.

Eric A. Schon Ph.D.

RG	Treatment strategies for human mitochondrial disease				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	
	\$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.

Rochester - University of Rochester Robert Dirksen Ph.D.

RG	Orai1 as a Therapeu	tic Target for Central Co	re Disease	
	\$100,000.00	8/1/2013	7/31/2014	Year 1
	\$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.

RG	Recruitment for HYP HOP	HYP HOP Phase III Trial in the Periodic Paralyses		
	\$.00	7/1/2012	6/30/2013	Year 5

Summary A clinical trial called HYP HOP is being conducted to see whether either of two drugs, acetazolamide, or dichlorphenamide, helps to decrease attacks of weakness in hyper-periodic paralysis and hypo-periodic paralysis and whether either one helps to prevent the permanent weakness that develops in these diseases. This can provide physicians with a standard treatment for the diseases. NIH has funded the study but additional funds are requested to help to complete the study.

Robert Griggs M.D.

SG	Novel Molecular M	echanisms of Neuromuscular	Disease: Implication	ons for Therapy
	\$10.000.00	7/1/2013	6/30/2014	Year 1

Summary This conference will bring new collaborative opportunities and substantive scientific information to neuromuscular investigators. It will provide senior investigators, NIH program staff, and patient advocacy group/foundation representatives opportunities to meet with junior/trainee investigators who are in the process of developing careers in clinical/translational investigation. Trainees will interact with leaders in the field as they discuss innovative methodology designed to meet the challenges of studies of uncommon diseases and provide perspective on clinical studies in various phases of development. Younger investigators will be profiled during a hot topics session. Furthermore, the poster sessions, the discussions after each session, the meals and other informal settings offer them a chance to question and to present their own ideas. We anticipate that there will be up to 150 attendees.

Araya Puwanant M.D.

CRTG Disease Progression and Physiological Changes in Myotonic Dystrophy \$90,000.00 7/1/2012 6/30/2013 Year 2

Year 3

\$.00 8/31/2013

Summary In recent years there has been progress in understanding why people with myotonic dystrophy develop muscle weakness and muscle stiffness (myotonia). As more is being learned about the disease, scientists are beginning to develop experimental treatments. It is possible that some of these treatments may progress from laboratory testing to testing in people. If that happens, there will be a need for neuromuscular physicians who have specialized training and experience with research on myotonic dystrophy. One of the goals of this training grant is to provide the applicant, Dr. Araya Puwanant, with training and supervised experience so that she can become a future leader of research on myotonic dystrophy and other muscle conditions. During her training, Dr Puwanant will take part in research studies to carefully chart the progression of myotonic dystrophy over time in a large group of the patients. This research can help to determine the best way to measure the severity of myotonic dystrophy. This information could be important in the future, to determine whether new treatments, if they are developed, are having a beneficial effect.

Jeffrey Statland M.D.

CRTG Clinical Trial Preparedness in Facioscapulohumeral Dystrophy \$90,000.00 7/1/2012 6/30/2013 Year 1 \$83,709.00 7/1/2013 6/30/2014 Year 2

Summary Recent advances have led to a unifying genetic model for facioscapulohumeral muscular dystrophy (FSHD). For the first time ever potential drug targets are being identified for therapy, so it is of vital importance that the clinical trial tools are in place and the next generation of clinical investigators trained to run trials in FSHD. The MDA Clinical Research Training Grant (CRTG) will enable me to work with Dr. Rabi Tawil at the University of Rochester to take a multi-tiered approach to develop reliable, patient relevant outcome measures for use in FSHD clinical trials. Our strategy utilizes existing data bases to gain a better understanding of the characteristic unique progression of disease in FSHD. In addition we are proposing two new projects: the first to develop a disease specific patient reported outcome measure and disease specific functional rating scale for use in clinical trials; and the second to develop a novel wireless gait and motion analysis system as a surrogate measure of strength for FSHD. Our goal in working with our national and international collaborators is to be ready to run a clinical trial for the first disease-directed therapy in FSHD within 3-5 years. At the end of my 2 year CRTG period I hope to have obtained continued funding for our projects, and the skills necessary to be an independent clinical investigator.

Al-Rabi Tawil M.D.

RG An Exploratory Study of Serum and Muscle Biomarkers in FSHD \$92,222.00 2/1/2013 1/31/2014 Year 1

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of **Summary** muscular dystrophy affecting 1:20,000 individuals. Recent research indicates that FSHD results from turning on expression of a gene (DUX4) usually silenced in adult muscle tissue. This gene is believed to control a group of genes involved in early cell growth and development. Activation of these genes in adult muscle may cause disease by being directly toxic to cells, interfering with normal cell function, or by inappropriately activating our own immune systems. Having identified potential therapeutic targets in FSHD, it becomes crucial to identify sensitive, disease-related biological markers (biomarkers) that can be easily measured in blood or muscle tissue to assess the effectiveness of future disease-modifying drugs. Indeed at the 2011 FSH Society International Workshop identifying useful biomarkers was one of the main priorities for the coming year. Recent studies have identified a subset of these abnormally expressed genes that were seen in FSHD but not healthy individuals making them ideal as potential biomarkers. We will examine muscle biopsies and serum from patients with FSHD for the presence of these potential biomarkers and compare the levels of expression to muscle pathology and clinical measures of disease severity. At the end of this study we hope to have promising biomarkers for future clinical trials.

Charles Thornton MD

RG	Models for therapeutic development in DM1				
	\$107,271.00	8/1/2012	7/31/2013	Year 1	
	\$107,271.00	8/1/2013	7/31/2014	Year 2	
	\$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	12/1/2012	11/30/2013	Year 1	
	\$306,000.00	12/1/2013	11/30/2014	Year 2	
	\$306,000.00	12/1/2014	11/30/2015	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.

Thurman Wheeler M.D.

RG	Progressive myopathy and therapeutic development in myotonic dystrophy type 1				
	\$132,000.00	8/1/2012	7/31/2013	Year 1	
	\$132,000.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Syracuse - SUNY Upstate Medical University Jeremy Shefner MD, PhD

HCT	ICT Multi-center, Randomized Controlled Study of Diaphragm Pacing for ALS			
	\$225,000.00	1/1/2013	12/31/2013	Year 2
	\$250,000.00	1/1/2014	12/31/2014	Year 3
	\$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.

NORTH CAROLINA

Chapel Hill - The University of North Carolina at Chapel Hill Joan M. Taylor Ph.D.

	- , -			
RG	Muscle developmen	t and repair mediated by	the BAR-containing Rl	no GAP, GRAF
	\$132,000.00	2/1/2013	1/31/2014	Year 1
	\$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 Medical Center Clayton S Owens MSLS

SG Third International Workshop for Glycosylation Defects in Muscular Dystrophies \$5,000.00 4/1/2013 4/19/2013 Year 1

Summary This international workshop focuses on a subset of muscular dystrophies characterized by glycosylation defects. It will bring together internationally renowned scientists and clinicians in muscle disease research to advance the understanding of muscular dystrophies associated with glycosylation defects. The workshop consists of 22 platform presentations comprising three general sessions: Glycosylation; AAV Therapy/Drug Discovery; and Clinical Management/Endpoints. The meeting intends to provide a unique platform to facilitate international collaboration in developing treatment plans and to define a strategy to achieve this goal.

Susan Sparks M.D., Ph.D.

RRG Longitudinal Assessment and Genetic Identification of Limb-Girdle Musc Dystrophy \$188,987.28 10/1/2012 9/30/2013 Year 1

Summary Limb-girdle muscular dystrophy (LGMD) is largely a descriptive term for a molecularly heterogeneous group of muscular dystrophies with onset in childhood or adulthood that is characterized by progressive muscle weakness. LGMD are classified into two groups based on the mode of inheritance, type 1 for autosomal dominant and type 2 for autosomal recessive. Each type is further subdivided depending on the molecular etiology, designated by a letter in the order they were discovered (i.e. LGMD1A-E and LGMD2A-N). Molecular clarification has resulted in the elucidation of common pathways of pathogenesis, as well as important differences between subtypes of LGMD. The community has identified lack of natural history studies as a major gap in our knowledge base and a significant barrier to the development of effective clinical trials in LGMD. In addition, the lack of validated clinical trial endpoints makes it near impossible to transition potential therapeutics through rigorous clinical trials into routine treatments for LGMD. This proposal aims to comprehensively evaluate individuals with genetically identified LGMD and follow potential outcome measures longitudinally in patients with LGMD. In addition, there is an aim to perform whole-exome sequencing on patients with clinically diagnosed LGMD without an identified genetic mutation.

Xiaohua Wu PhD, MD,

RG Enhancing Laminin Binding to Treat Muscular Dystrophies

\$106,015.00	2/1/2012	1/31/2013	Year 2
\$.00	2/1/2013	1/31/2014	Year 3

Summary The long term goal of our research is to discover effective treatments for muscular dystrophies. Currently, there is no effective treatment for any forms of MD. A number of muscular dystrophies (MD) are associated with compromised linkage between basement membrane and myofibers due to various genetic defects. The linkage plays a key role in muscle function. Enhance the linkage through via over expression certain genes have been proved useful in MD mouse models. In this project, we plan to identify small compounds that can enhance the linkage. We recently developed a cell-based assay. We plan to screen large number of small compounds (100,000)to identify the compounds Further, we plan to test the identified compounds in cell culture and MD mouse models to develop drug for MDs.

Durham - Duke University

Charles Alan Gersbach Ph.D.

RG	Genetic Correction of Duchenne Muscular Dystrophy with Engineered Nucleases					
	\$100,000.00	8/1/2013	7/31/2014	Year 1		
	\$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.

Durham - Duke University Medical Center

Moon-Chang Choi Ph.D.

DG HDAC4 is involved in muscle regeneration; New therapeutic avenue for DMD disease

\$60,000.00	8/1/2013	7/31/2014	Year 1
\$60,000.00	8/1/2014	7/31/2015	Year 2

Summary Muscular dystrophies including DMD (Duchenne muscular dystrophy) result in progressive muscle wasting, exceeding the regenerative capacity of muscle. While damaged muscle should be properly removed and regenerated by the muscle repair system, DMD muscle has poor and impaired regenerative capacity. Accordingly, deciphering the molecular pathways and components that regulate muscle regeneration could hold the key to the development of effective therapy for muscular dystrophies. We recently identified HDAC4 as a key factor for the muscle atrophy program in response to denervation. Here, we propose that HDAC4 is also involved in muscle repair program. We found that HDAC4 is induced and prominently localized both in satellite cells and in newly regenerating fibers following muscle damage. Moreover, we found that HDAC4 is required for satellite cell proliferation induced by damage. Based on these preliminary observations, in this proposal, we will investigate how HDAC4 regulates muscle regeneration and if loss of HDAC4 exacerbates dystrophic phenotypes in mdx

mice. The successful completion of this study would provide insights into novel therapeutic approaches for DMD.

Michael Hauser Ph.D

RG	The Genetic Basis of Autosomal Dominant LGMD			
	\$127,952.00	2/1/2012	1/31/2013	Year 2
	\$127,952.00	2/1/2013	1/31/2014	Year 3

Summary We have collected a number of families in which limb girdle muscular dystrophy affects multiple family members. We will analyze the DNA from those affected individuals in order to find the specific mutations (DNA changes) that cause disease. The identity of these mutations and the genes that they affect will improve our understanding of the way these diseases damage muscle tissue, and will allow the development of new therapeutic strategies. Relevance to MDA Development of new treatments for autosomal dominant muscular dystrophies requires a clear understanding of the causative mutations and the mechanism by which those mutations cause disease. This proposal builds on a previously funded MDA grant 4090 "Genetic Studies in Unlinked Muscular Dystrophies" which supported the linkage analysis of a number of dominant LGMD families. That work successfully linked multiple pedigrees. In the present proposal, we will continue analysis of these families to identify the causative mutations.

Dwight Koeberl M.D., Ph.D.

RG	Enhanced muscle-targeted gene therapy for Pompe disease				
	\$126,906.00	8/1/2012	7/31/2013	Year 1	
	\$126,906.00	8/1/2013	7/31/2014	Year 2	

Summary The focus of this proposal is glycogen storage disease type II (GSD-II; Pompe disease; MIM 232300), which results from the inherited deficiency of lysosomal acid alpha-glucosidase (GAA; acid maltase; EC 3.2.1.20). Pompe disease is characterized by the massive accumulation of lysosomal glycogen in striated muscle with an accompanying disruption of cellular functions. Enzyme replacement therapy (ERT) is available for Pompe disease; however, the ERT dosages range up to 100-fold greater than those in other lysosomal disorders. High dosage requirements can be attributed to the need to treat the very large muscle mass and to limited receptor-mediated uptake of the therapeutic protein in Pompe disease. We will investigate Specific Aim 1: To evaluate the effect of immune tolerance in mice with Pompe disease; and Specific Aim 2: To evaluate the effect of increased CI-MPR in a human Pompe muscle model. The proposed development of new therapy in Pompe disease, including adjunctive therapy and immunomodulatory gene therapy, will have significant public health impact with implications for the treatment of both Pompe disease and the muscular dystrophies.

OHIO Cincinnati - University of Cincinnati Tom Thompson Ph.D.

RG	Structural studies of myostatin inhibitors				
	\$110,000.00	8/1/2012	7/31/2013	Year 1	
	\$110,000.00	8/1/2013	7/31/2014	Year 2	
	\$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	8/1/2012	7/31/2013	Year 1	
	\$130,000.00	8/1/2013	7/31/2014	Year 2	
	\$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 - Nationwide Childrens Hospital Kevin M Flanigan M.D.

SG	Nationwide Children's Hospital/Wellstone/Ohio State University Myology Course			
	\$10,000.00	12/2/2012	8/30/2013	Year 2

Summary We propose a one-week intensive course directed toward both clinically and laboratoryoriented post-doctoral trainees. The program has been developed by Dr. K. Flanigan, Professor of Pediatrics and Neurology at the Ohio State University, and Dr. D. Guttridge, Assoc. Professor of Cell Biology and Genetics at OSU. Faculty include recognized leaders in neuromuscular care and research, mostly among members of the Nationwide Children's Hospital/OSU Muscle Group. The target audience is international and we have received requests for enrollment from leading training programs in the United States and South America. Each morning, attendees will receive lectures on neuromuscular disease genetics and pathophysiology. Each afternoon clinical trainees will receive didactic and hands-on training including but not limited to neuromuscular pathology, methods of clinical assessment, and cardiorespiratory care. Laboratory trainees will have a "wet lab" experience with hands-on training in muscle cell culture, muscle electrophysiology, and muscle precursor cell isolation. Each day will close with the two groups joining for a keynote lecture. The program is designed to provide trainees with an integrated and intensive introduction to principles of neuromuscular disease. It is modeled on the highly successful Summer School of Myology held each July at the Institute of Myology, Paris. That program now attracts over fifty students annually, and we anticipate an eventual similar demand here.

Columbus - Research Institute at Nationwide Children's Hospital Scott Q. Harper Ph.D.

RG	Development of An Inducible FSHD Mouse Model			
	\$109,494.00	2/1/2012	1/31/2013	Year 1
	\$99,222.00	2/1/2013	1/31/2014	Year 2
	\$108,748.00	2/1/2014	1/31/2015	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.

Jerry Mendell M.D.

MVP	Improve Limb Strength by Vascular Delivery of the Alpha-Sarcoglycan Gene in
LGMD2D	

\$.00 1/1/2012 8/31/2013 Year 3

Summary This grant proposal describes the entire step-by-step program inclusive of all phases of the plan including the clinical trial for gene therapy for LGMD2D. For this specific grant request to MVP we are asking for funds to perform the toxicology-biodistribution study and obtain the IND (Specific Aims 1 and 2). The GMP grade vector production and execution of the clinical trial are described in Specific Aims 3 and 4 that represent a follow up study (funds not requested now). Two points are important to emphasize: 1) the support requested in this proposal will have clinically meaningful outcomes with gene delivery to the thigh muscles (quadriceps); 2) accomplishing this goal will permit the follow up step of vascular delivery to the entire lower limb. Given that there is no heart disease in LGMD2D, full limb delivery would mean a dramatic save for the patients with this disease.

Jerry Mendell M.D.

RG	Supplemental Support for AVI-4658 Phase II Clinical Trial				
	\$.00	7/31/2012	9/30/2013	Year 2	
	\$.00		10/1/2013	Year 3	

Summary This study is to assess the efficacy, safety, and tolerability of AVI-4658 (Eteplirsen) in both 50.0 mg/kg and 30.0 mg/kg doses administered over 24 weeks in subjects diagnosed with Duchenne muscular dystrophy (DMD). This request is for funding for supplemental support for this trial. Funds requested will go towards support of patient travel expenses for the 24 weekly visits to our clinic, and towards support of personnel and costs of supplies and reagents for analysis of dystrophin expression in muscle biopsies. Assessing dystrophin expression is a critical outcome measure for the clinical trial.

Columbus - The Ohio State University

Noah Weisleder Ph.D.

RG	Protein therapy targeting limb girdle muscular dystrophy				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	
	\$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 (OSU) Jill Rafael-Fortney Ph.D.

RG Investigation of a new treatment target for heart failure in muscular dystrophy \$126,923.00 1/1/2012 8/31/2013 Year 3

Summary At least 95% of Duchenne muscular dystrophy (DMD) patients develop cardiomyopathy. As therapies to protect respiratory function improve, DMD patients live longer, and the chance of heart failure will approach 100%. Supporting this prediction, Becker muscular dystrophy patients with milder

skeletal muscle disease all develop severe cardiac disease. We have discovered that the claudin-5 protein is deficient specifically in heart muscle cells in a muscular dystrophy mouse model that exhibits heart failure. Claudin-5 reductions occur in a time-frame that makes it an excellent candidate as a therapeutic target. We have also identified specific reductions of claudin-5 in at least 60% of patients with heart failure, demonstrating the clinical relevance of this protein and further supporting that claudin-5 may be a key "switch" from many forms of cardiomyopathy to progression of heart failure. Claudin-5 therefore represents a novel potential therapeutic target for treatment of DMD related cardiomyopathy and heart failure. In this study, we will define the mechanisms specific to claudin-5 deficiency, determine claudin-5 levels in other forms of muscular dystrophy, and determine whether exogenous claudin-5 expression is sufficient to prevent heart failure in muscular dystrophy mouse models. This study will directly address the potential of a novel protein as a treatment target for prevention of heart failure in muscular dystrophy patients.

Columbus - The Research Institute at Nationwide Children's Hospital Kevin M Flanigan M.D.

SG 2nd Annual Nationwide Children's Hospital/Wellstone/OSU Myology Course \$23,048.00 8/26/2013 8/30/2013 Year 1

Summary We propose a second annual one-week intensive course directed toward both clinically and laboratory-oriented post-doctoral trainees. The program has been developed by Dr. K. Flanigan, Professor of Pediatrics and Neurology at the Ohio State University, and Dr. D. Guttridge, Assoc. Professor of Cell Biology and Genetics at OSU. Faculty include recognized leaders in neuromuscular care and research, mostly among members of the Nationwide Children's Hospital/OSU Muscle Group. The target audience is international and we have received requests for enrollment from leading training programs in the United States, Europe, and South America. Each morning, attendees will receive lectures on neuromuscular disease genetics and pathophysiology. Each afternoon clinical trainees will receive didactic and hands-on training including but not limited to neuromuscular pathology, methods of clinical assessment, and cardiorespiratory care. Laboratory trainees will have a "wet lab" experience with hands-on training in muscle cell culture, muscle electrophysiology, and muscle precursor cell isolation. Each day will close with the two groups joining for a keynote lecture. The program is designed to provide trainees with an integrated and intensive introduction to principles of neuromuscular disease. It is modeled on the highly successful Summer School of Myology held each July at the Institute of Myology, Paris.

Paul Martin Ph.D.

RG	Protein-based GALGT2 therapies for Duchenne muscular dystrophy				
	\$132,000.00	8/1/2012	7/31/2013	Year 2	
	\$132,000.00	8/1/2013	7/31/2014	Year 3	

Summary This proposal seeks to develop two new therapies for the treatment of Duchenne muscular dystrophy (DMD). The investigators have shown that overexpression of a naturally occurring gene, called Galgt2, in skeletal muscles can inhibit the development of disease in the mdx mouse model for DMD. Here, they seek to translate this idea into a therapy for DMD. The approach is to test two new protein therapies in a DMD animal model that would increase the expression of Galgt2. One approach is to add a factor that stimulates the ability of skeletal muscles to increase their own expression of Galgt2. The other approach is to engineer a Galgt2 protein that can cross into muscle cells from the serum to directly increase expression levels. Because overexpression of Galgt2 has been shown to be therapeutic in other models of muscular dystrophy (Congenital muscular dystrophy 1A and Limb Girdle Muscular Dystrophy 2D), any approach shown to work in DMD may also apply to these other forms of the disease.

Jerry Mendell M.D.

CRNG	MDA Clinical Network					
	\$306,000.00	11/1/2012	10/30/2013	Year 1		
	\$306,000.00	11/1/2013	10/30/2014	Year 2		
	\$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.

Toledo - The University of Toledo Kenneth Hensley Ph.D.

RG	Targeting CRMP2 to Treat Motor Neuron Disease				
	\$110,679.00	2/1/2012	1/31/2013	Year 1	
	\$108,868.00	2/1/2013	1/31/2014	Year 2	
	\$108,606.00	2/1/2014	1/31/2015	Year 3	

Summary Amyotrophic lateral sclerosis (ALS) is a degenerative disease in which the neural circuits (axons) that connect the brain to the spinal cord, and the spinal cord to the muscle, wither away. Recent findings from our own laboratory, and other research groups, suggest that ALS actually begins near the junction of nerve and muscle (the neuromuscular junction or NMJ). We hypothesize that molecules called semaphorins that are expressed inappropriately near the NMJ, signal neuron axons to retract away from the muscle and "collapse" backward toward the spinal cord. Our research implicates a central protein called collapsin response mediator protein-2, or CRMP2, in this process of axon degeneration. We have invented and patented small molecule compounds called lanthionines that bind CRMP2 and inhibit or reverse CRMP2-dependent axonal degeneration. We are also researching antibodies that could be administered to ALS patients in order to block semaphorin binding to neural receptors, hence preventing inappropriate activation of CRMP2 pathways. We will test three complementary but distinct pharmacological approaches to interrupting CRMP2-dependent axon degeneration in the G93A-SOD1 transgenic mouse model of ALS. Success in this project would launch a new drug development program centered on CRMP2 function-boosting therapeutics, the long-term goals of which would be creation of investigational new drug (IND) application(s) and ultimately, clinical trials against ALS.

OREGON

Portland - Oregon Health & Science University paul brehm Ph.D

RG	Use-dependent fatigue in muscle rapsyn myasthenic syndrome is presynaptic				
	\$117,216.00	8/1/2012	7/31/2013	Year 1	
	\$117,216.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Michael W. Linhoff Ph.D.

DG	Presynaptic structure and molecular composition in a zebrafish myasthenia model					
	\$59,972.00	2/1/2013	1/31/2014	Year 1		
	\$59,972.00	2/1/2014	1/31/2015	Year 2		

Summary A common cause of congenital myasthenic syndrome (CMS) is mutation of the RAPSN gene. Rapsyn is a cytoplasmic, membrane associated protein that is required for acetylcholine receptor (AchR) clustering at the neuromuscular junction (NMJ). Our lab previously identified a mutant fish line, twitch once, that exhibits use-dependent fatigue similar to patients with CMS, and the mutation underlying the behavioral phenotype was determined to be in the gene encoding rapsyn. For both CMS patients and twitch once fish, impaired synaptic transmission is assumed to be due to deficits in AchR clustering at the synapse. Our lab has found that twitch once mutants unexpectedly display significant presynaptic dysfunction. Paired motor neuron-muscle recordings show profound synaptic depression in rapsyn mutants, and functional imaging studies using the exocytosis indicator, synaptopHluorin, reveal a population of synaptic vesicles that is reluctant to release. I am using array tomography to develop high-resolution, three dimensional models of the zebrafish NMJ to address the molecular composition of synapses in wild type and mutant zebrafish lines. I will capitalize on the use of zebrafish and new high resolution imaging technologies to elucidate mechanisms underlying synapse dysfunction in the twitch once motility mutant.

PENNSYLVANIA

Philadelphia - Philadelphia Health and Education corporation d/b/a Drexel University College of Medicine

Terry Heiman-Patterson MD

RG	Identification of modifying genes in murine models of ALS				
	\$110,000.00	2/1/2012	1/31/2013	Year 1	
	\$110,000.00	2/1/2013	1/31/2014	Year 2	
	\$110,000.00	2/1/2014	1/31/2015	Year 3	

A mouse model for ALS has been created that carries the human form of an ALS gene Summary (huSOD1-transgenic mice). Our labs along with others have shown that disease severity in these transgenic mice depends on their genetic background. Thus, transgenic mice from the ALR, NOD.Rag1KO, FVB, SJL or C3H strains display a more severe phenotype than (B6xSJL) transgenic mice or transgenic mice from B6, B10, BALB/c, and DBA/2J backgrounds. We propose that the comparison of these mouse models will aid in identification of genes that can modify disease. In fact, the DUCOM and Jackson Labs have each identified a region on chromosome 17 that modifies disease severity on the SJL and ALR backgrounds. This application is a collaborative project (DUCOM, Jackson Labs) directed at finding the gene in the Chr 17 interval that affects severity of disease in the G93A SOD1 mouse model of ALS. The goals of this application are to validate that the region of Chr 17 does modify disease, to identify the responsible gene within the region, and to test whether the gene can also affect severity in other models of motor neuron disease. Identification of modifier genes will highlight intracellular pathways already suspected to be involved in motor neuron degeneration or point to new pathways and processes that have not yet been considered. Most importantly, identified modifier genes provide new targets for the development of therapies.

Philadelphia - Temple University School of Medicine

Young-Jin Son Ph. D

RG	Muscarinic regulation of muscle growth and atrophy				
	\$125,000.00	2/1/2012	1/31/2013	Year 2	
	\$125,000.00	2/1/2013	1/31/2014	Year 3	

Stable maintenance of connections between nerves and muscles and of muscle size is critical for normal neuromuscular activity in adulthood and is important in the initiation and progression of numerous neuromuscular disorders. We propose to study whether muscarinic acetylcholine receptors constitute a novel receptor system that is used by neuron and muscle to monitor neural activity and to maintain stable nerve-muscle connections and solid muscle mass. In addition, we will determine if muscle atrophy can be prevented by treatments that directly target muscarinic receptors. Our studies will provide insights that may prevent synaptic loss and muscle atrophy, and has the potential to develop new strategies for treating a broad range of neuromuscular disorders.

Philadelphia - The Children's Hospital of Philadelphia Masahiro Iwamoto Ph.D.

RG	Intervention of muscular dystrophy by selective RARgamma agonist				
	\$135,000.00	2/1/2013	1/31/2014	Year 1	
	\$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.

David Lynch MD, PhD

RG	Insulin resistance in Friedreich ataxia			
	\$101,111.00	8/1/2012	7/31/2013	Year 2

Summary Friedreich ataxia (FRDA) is a progressive neuromuscular, neurodegenerative disease of children and young adults. One of the most devastating, but as yet poorly understood, non-neurological features of FRDA is the high prevalence of diabetes. This grant will attempt to understand the features contributing to diabetes in FRDA, and thereby reveal new therapeutic strategies for the overall disease.

Philadelphia - The Trustees of the University of Pennsylvania Tathagata Chaudhuri Ph.D.

DG	Matrix Conditioning of Mesenchymal Stem Cells to Rescue Muscular Dystrophies					
	\$60,000.00	8/1/2012	7/31/2013	Year 1		
	\$60,000.00	8/1/2013	7/31/2014	Year 2		
	\$60,000.00	8/1/2014	7/31/2015	Year 3		

Summary The specific goal of this study is to develop a novel technology which will direct human Mesenchymal Stem Cells (MSCs) to form muscle cells and therefore repair damaged skeletal muscle in muscular dystrophies, particularly, Duchenne Muscular Dystrophy (DMD). MSCs are commercially available and can be engineered to differentiate into muscle and our objective is to show that matrix based-conditioning of these MSCs can induce differentiation into muscle like cells. First, we will test if these preconditioned MSCs are capable of rescuing muscle defects and lead to myogenesis when injected into the damaged muscles of mdx mice, the mouse model of DMD. Secondly, to examine if this work can be clinically translated, we will also apply the same technology in the golden retriever muscular dystrophy (GRMD) dogs which exhibit a more severe dystrophic phenotype and closely resembles the human condition. We will determine if dog MSCs derived from GRMD dogs can also be programmed by matrix specification into a myogenic fate, similar to human MSCs. Finally, we will

engineer these dog MSCs to express dystrophin, commit them into a myogenic lineage and inject them back into the same donor GRMD dog to examine if muscle repair and regeneration occur. These goals will determine if matrix elasticity alone can induce both human and canine MSCs to be committed into a myogenic lineage and whether this approach of utilizing preconditioned cells can be used for cellular therapy of muscular dystrophies.

Tejvir S. Khurana MD, Ph.D.

RG	Utrophin upregulation via microRNA repression as a therapy for DMD				
	\$126,500.00	8/1/2012	7/31/2013	Year 2	
	\$126,500.00	8/1/2013	7/31/2014	Year 3	

Summary Utrophin is highly related to the dystrophin gene. It is of great therapeutic interest since increasing its production in muscles can compensate for the lack of dystrophin in animal models of DMD. We have found that utrophin is in a state of repression and that a class of molecules called microRNA's cause the repression. We will develop methods to "repress the repressors" and hence achieve Utrophin upregulation. These approaches will be tested in the mdx mouse model of DMD.

Jon Martin Lindstrom Ph.D.

RG	Specific Immunosuppression of EAMG				
	\$150,000.00	2/1/2012	1/31/2013	Year 2	
	\$150,000.00	2/1/2013	1/31/2014	Year 3	

Summary We have developed a way to specifically suppress the autoimmune response which causes myasthenia gravis in the animal model of this disease. We want to perfect this therapy before trying to treat naturally occurring myasthenia gravis in dogs, cats or humans. Further, we want to investigate the mechanisms by which this therapy works.

James Shorter Ph.D.

RG	Generating Therapeutic Protein Disaggregases for Amyotrophic Lateral Sclerosis				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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	8/1/2013	7/31/2014	Year 1	
	\$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				
	\$91,553.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Philadelphia - Thomas Jefferson University Dena Jacob Ph.D.

DG Multi-drug resistance in amyotrophic lateral sclerosis: implications for therapy \$60,000.00 7/1/2012 9/30/2013 Year 3

Despite numerous drug trials to cure the mouse that models amyotrophic lateral *Summary* sclerosis (ALS), attempts have so been unsuccessful. Multi-drug efflux transporters are proteins that influence the drug response by pumping drugs out of cells. The transporter P-glycoprotein (P-gp) recognizes a broad range of drugs and is normally found in cells of the blood-brain and blood-spinal cord (BBB/BSCB) barriers. Under certain neuropathological conditions P-gp is also expressed in affected neural tissue, thus further limiting drug penetration. A toxic buildup of the excitatory neurotransmitter glutamate occurs in ALS mice due to reduced glutamate transporter function, and we previously identified the drug nordihydroguaiaretic acid (NDGA) as a potent and specific glutamate transport activity enhancer. Tests of this drug in the ALS mouse revealed a consistent yet transient up-regulation of glutamate uptake. Interestingly, ALS mice also displayed a disease-driven increase of spinal cord P-gp expression. Together with the evidence that NDGA could be a potential substrate for P-gp, I hypothesize that the therapeutic failure of NDGA in ALS mice could be accounted for by acquired, P-gp mediated, pharmacoresistance. Using both genetic and pharmacological approaches to inhibit P-gp, I will test the true therapeutic efficacy of NDGA. Further, I will use both in vitro and in vivo approaches to examine how ALS affects the normal localization and expression patterns of P-gp.

RHODE ISLAND

West Kingston - Gordon Research Conferences Peter David Currie PhD

SG Myogenesis Gordon Research Conference and Myogenesis Gordon Research Seminar

\$10,000.00 7/1/2013 7/31/2013 Year 1

Summary The central objective of the 2013 Myogenesis Gordon Research Conference (GRC) entitled "Models and Mechanisms in Myogenesis" is to stimulate discussion, scientific interchange and insight into the factors affecting muscle tissue formation and repair during muscle development and disease. The participants present new, unpublished research on a wide range of topics including muscle specification, cell interplay during muscle development, evolutionary mechanisms of muscle formation, insights into muscle stem cells, and regeneration and there will be a strong emphasis on advanced animal disease modelling to examine the genetic and morphogenetic basis for muscle formation and repair. There will be several sessions that deal directly with research targeted at generating treatments for a range of muscular dystrophies. These topics are central to the MDAs mission of funding research that seeks to understand the causes of, and effective treatments for, neuromuscular diseases. The

meeting will bring together 50 speakers that represent critical areas of striated muscle research with a total of 180 participants for a five-day conference. A new component of the 2013 meeting will be the Gordon Graduate and Post-doctoral Seminar on Myogenesis, held immediately before the main meeting. Students and post-doctoral researchers will interact and present their research in an exciting, unintimidating environment consisting wholly of their peers before attending the more traditional GRC.

TENNESSEE

Memphis - St. Jude Children's Research Hospital Brian David Freibaum Ph.D

DG Characterizing the role of TDP-43 in ALS

\$60,000.00 7/1/2012 6/30/2013 Year 3

Summary TDP-43 is the major disease protein in both sporadic and familial amyotrophic lateral sclerosis (ALS). In diseased motor neurons, TDP-43 redistributes from the nucleus to the cytoplasm of neurons where it forms aggregates within the neuron. Additionally, dominantly inherited mutations found within the TDP-43 gene have been associated with both sporadic and familial ALS. It is not yet known how TDP-43 leads to disease. I hypothesize that TDP-43 leads to RNA mediated toxicity within the cytoplasm of affected neurons through a toxic gain of function mechanism. I will identify which regions of TDP-43 mediate disease by using model systems in human cells and Drosophila (fruit fly). Additionally, I seek to understand the role TDP-43 plays in disease by identifying proteins that physically interact with TDP-43. Finally, I will use a targeted genetic screen to identify additional proteins that play a role in mediating TDP-43 toxicity in neurons. Understanding the mechanism by which TDP-43 leads to the development of ALS will generate new avenues of research into ALS therapies. Understanding how TDP-43 leads to ALS will provide a more targeted approach to the development of new therapies. Additionally, identification of novel proteins that are required for TDP-43 mediated toxicity will provide novel targets for future therapies or potential drug screens.

Hong Joo Kim Ph.D.

DG	Characterizing the role of pathogenic mutations of hnRNPs in IBMPFD and ALS				
	\$60,000.00	2/1/2013	1/31/2014	Year 1	
	\$60,000.00	2/1/2014	1/31/2015	Year 2	
	\$60,000.00	2/1/2015	1/31/2016	Year 3	

Multisystem proteinopathy (MSP), formerly known as IBMPFD, is an inherited *Summary* 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.

J. Paul Taylor MD, PhD

RG	The molecular pathogenesis of spinobulbar muscular atrophy				
	\$110,000.00	2/1/2012	1/31/2013	Year 2	
	\$110,000,00	2/1/2013	1/31/2014	Year 3	

Summary In prior research funded by the MDA, we developed a fruit fly model of spinobulbar muscular atrophy (SBMA) and used this model to determine how mutations in AR lead to the death of neurons and muscle atrophy observed in this disease. Specifically, we have determined that toxicity occurs only when mutant AR enters the cell nucleus and binds DNA. Importantly, we have determined that toxicity in this invertebrate model is mediated by a small interaction surface on AR called "AF2". We have also determined that toxicity is strongly enhanced by a chemical modification called "sumoylation". These discoveries reveal an opportunity for therapeutic intervention by using small molecule inhibitors of AF2 and sumoylation. Here we propose to test the validity of these therapeutic targets in a novel mouse model of SBMA. We will generate transgenic mice expressing normal and mutant forms of AR, including mutant forms that are defective in DNA binding, incapable of undergoing sumoylation, or have the AF2 surface disrupted. Evaluation of these animals will reveal the potential of AF2 and sumoylation as targets for therapy. In parallel, we will identify small molecule inhibitors of these targets using our fly model, with the potential of testing these inhibitors in mouse following validation of the relevant targets.

TEXAS

Dallas - UT Southwestern Medical Center Steve Cannon MD, PhD

RG	Disease pathogenesis and modification in a mouse model of HypoPP				
	\$132,749.00	8/1/2012	7/31/2013	Year 2	
	\$132,749.00	8/1/2013	7/31/2014	Year 3	

Summary Periodic paralysis is a rare disorder of skeletal muscle wherein affected individuals have recurrent attacks of severe weakness, lasting for hours to days. These episodes are caused by a temporary disruption of muscle electrical excitability, which serves as the trigger to initiate contraction. In addition, some patients develop a late-onset permanent weakness in the legs that may prevent independent ambulation. The gene defects that cause periodic paralysis were identified over a decade ago, and yet we still do not understand mechanistic basis for the attacks or weakness nor is there an effective treatment. With prior support from the MDA, we generated a mouse model for periodic paralysis caused by a mutation in the sodium channel gene. We will now use this unique experimental tool to gain insights on how the behavior of mutant channels is altered, to explore how defects in channel function cause attacks of weakness, and to test potential therapeutic interventions to modify disease severity.

Jeffrey Leigh Elliott M.D.

RRG Animal models in ALS

\$77,021.00 6/1/2012 5/31/2013 Year 1

Summary Amyotrophic Lateral Sclerosis (ALS) is a progressive neurological disorder characterized by weakness affecting limb, bulbar and respiratory functions. Most cases of ALS are sporadic, but about 5%-10% of cases are hereditary. The cause of sporadic ALS is unknown, but there is good recent evidence to indicate an important role for the protein TDP-43. The mechanism underlying TDP-43 induced ALS is not clear. TDP-43 is normally found in all cells and appears to be critical for cell survival based on studies in mice with loss of TDP-43 protein. One of the normal functions of TDP-43 is to bind mRNA that codes for proteins and regulates the splicing of mRNA into mature transcripts. TDP-43 can bind many RNA species, and there are some identified targets that may be important in neurodegenerative disorders. In this proposal we wish to see if TDP-43 overexpression in mice that develop motor neuron disease will affect the splicing of sortilin1 mRNA. Sortilin1 is a receptor protein important in intra-cellular trafficking or targeting. Splicing of sortilin1 mRNA will be assessed in spinal cord, skeletal muscle and fat tissues longitudinally as the mice develop weakness. Understanding the normal function of TDP-43 and identifying its important targets in live animals will allow for the recognition of pathways that may be amenable to therapies.

Jeffrey Leigh Elliott M.D.

RRG A role for TDP-43 in regulating metabolism

\$10,192.69 3/1/2013 2/28/2014 Year 1

Summary Weight loss and muscle atrophy are frequently recognized clinical features of ALS, although the molecular basis for these abnormalities is not clear. Changes in the TDP-43 protein that is linked to both familial and sporadic ALS may have direct effects on muscle metabolism. In this project, we will test the hypothesis that TDP-43 has a direct effect on skeletal muscle metabolism that contributes to the metabolic derangements observed in patients with ALS.

Jeffrey Leigh Elliott M.D.

RRG Animal Models of ALS Cachexia

\$15,000.00 6/1/2013 5/31/2014 Year 1

Summary In the ALS clinic, we strive to prevent weight loss in patients knowing that the success translates into better quality of life and improved survival. We usually recommend increased caloric intake. However as calories from fat, protein, or carbohydrate may not be of equal value in preventing the cachexia, particularly if specific metabolic homeostatic pathways are perturbed in TDP-43 related disease, it is unclear from what source calories should be most encouraged. Knowledge from this project will be useful for guiding the best dietary strategies in disease. Clinical trials could readily be established to confirm the animal findings in human beings, allowing for the chance to readily improve patient outcomes.

Ronald Haller M.D.

RG Impaired Oxidative Capacity in McArdle Disease: Causes and Treatment

\$127,700.00 1/1/2012 6/30/2013 Year 3

Summary This study will investigate the metabolic basis of limited muscle oxidative capacity in McArdle disease and will attempt to determine the mixture of dietary carbohydrate, protein. fats, and nutritional supplements that best compensate for this biochemical defect to provide optimal exercise capacity in affected patients.

Peter Robin Hiesinger Ph.D.

RG	A Drosophila Model for Charcot-Marie-Tooth 2B Disease			
	\$100,000.00	8/1/2013	7/31/2014	Year 1
	\$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 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.

Douglas Millay Ph.D.

DG	Molecular control of mammalian myoblast fusion				
	\$60,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Galveston - The University of Texas Medical Branch at Galveston Premkumar Christadoss M.B.B.S

RG	Complement siRNA gene therapy for myasthenia gravis				
	\$130,000.00	2/1/2012	1/31/2013	Year 1	
	\$130,000.00	2/1/2013	1/31/2014	Year 2	
	\$130,000.00	2/1/2014	1/31/2015	Year 3	

Summary Genetic deficiency of specific complement components may not reflect the exact roles played by these factors during the development of autoimmune disease. To identify the specific functions executed by the complement system in the pathogenesis of a classical antibody mediated autoimmune disease like experimental autoimmune myasthenia gravis (MG), we will treat mice with MG with small interfering RNAs (siRNAs) targeting different complement factors. This novel approach will enable us to accurately identify complement-mediated immune functions and to establish novel complement siRNA based gene therapy for myasthenia gravis and other complement mediated neuromuscular diseases.

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	8/1/2013	7/31/2014	Year 1	
	\$100,500.00	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.

Susan Hamilton Ph.D.

RG	Central Core Disease and Mitochondrial Dysfunction				
	\$125,000.00	8/1/2012	7/31/2013	Year 2	
	\$125,000.00	8/1/2013	7/31/2014	Year 3	

Summary Central Core Disease is a dominantly inherited myopathy characterized by metabolically inactive regions in the center of the muscle fibers. Mitochondria, the energy producers of the cell, are

destroyed in the core regions. We will define the mechanisms of mitochondrial destruction in Central Core Disease and identify new therapeutic targets for CCD and other mitochondrial myopathies.

Xander H.T. Wehrens M.D., Ph.D.

RG	Mechanisms underl	ying arrhythmias and hear	t failure in Muscular [Dystrophy
	\$104,500.00	2/1/2012	1/31/2013	Year 2
	\$104,500.00	2/1/2013	1/31/2014	Year 3

Summary Patients with Duchenne muscular dystrophy (DMD) often develop heart failure and ventricular arrhythmias, both associated with sudden death. It has become clear that abnormal calcium cycling within the heart muscle cells plays a critical role in causing weakened cardiac contractility and arrhythmias which may both lead to sudden death. Our studies suggest that enhanced activity of an enzyme called 'calcium/calmodulin-dependent protein kinase II' (CaMKII) affects intracellular calcium channels such that they become leaky and interfere with normal calcium cycling. Moreover, increased oxidative stress may further potentiate the effects of CaMKII on abnormal calcium channels. Our lab will study several lines of genetically altered mice, in which CaMKII activity is inhibited, or the intracellular calcium release channels have been made resistant to the effects of CaMKII, in order to elucidate the detailed molecular mechanisms underlying calcium handling dysfunction. Using a laser confocal microscope, calcium fluxes will be studies in heart muscle cells isolated from these mouse models. Moreover, we will test the effects of pharmacological inhibitors of CaMKII and reactive oxygen species as candidates for the drug treatment of arrhythmias and heart failure in muscular dystrophy. Our studies will likely provide very specific clues about novel therapeutic targets for the development of drugs for the treatment of heart disease in patients with Duchenne muscular dystrophy.

Lee-Jun C. Wong Ph.D.

RG Innovative one-step diagnosis of complex mitochondrial disorders \$137,000.00 7/1/2012 6/30/2013 Year 3

Mitochondria are the cellular organelles where energy currency, ATP, is produced. Summary Diagnosis of mitochondrial disorder is very difficult because the disease itself can present in many different forms, including muscle weakness, exercise intolerance, ophthalmoplegia, sensorineural deafness, seizures, ataxia, movement disorder, and stroke like episodes. In addition to the small mitochondrial genome, as many as 1500 nuclear-encoded proteins are targeted to mitochondrion. Molecular defects in any of these genes can potentially cause mitochondrial disorder, which predominantly has neuromuscular features. Currently, the diagnosis is based on the gold standard of sequence analysis gene by gene. It is tedious, expensive, time consuming, and is only for the detection of point mutations. Other methods will have to be used for the detection of large deletions and copy number changes. We propose to establish a one-step novel technology that would allow us to detect point mutations and deletions in both nuclear and mitochondrial genomes, with simultaneous detection and estimation of heteroplasmic mitochondrial DNA point mutations or deletions, as well as mitochondrial DNA depletion. The availability of a one-step diagnostic approach is particularly important since mitochondrial myopathy accounts for a large proportion of patients, from children to adults, with muscular dystrophy. Prompt definitive diagnosis is essential for proper patient management, treatment, and genetic counseling.

Houston - The Methodist Hospital Research Institute Stanley Appel MD

RG Immune Mechanisms in Amyotrophic Lateral Sclerosis

\$110,000.00 7/1/2012 6/30/2013 Year 3

Summary Neuroinflammation is a pathological hallmark in amyotrophic lateral sclerosis (ALS), and is characterized by activated microglia and infiltrating T cells at sites of neuronal injury. In ALS, neurons do not die alone; neuronal injury is non cell-autonomous and depends on a well-orchestrated dialogue in which neuronally secreted misfolded proteins activate microglia and initiate a self-propagating cycle of neurotoxicity. Diverse populations and phenotypes of CD4+ T cells crosstalk with microglia, and depending on their activation status, influence this dialogue and promote neuroprotection or

neurotoxicity. A greater understanding of the T cell population that mediates these effects, as well as the molecular signals involved should provide targets for neuroprotective immunomodulation to treat this devastating neurodegenerative disorder

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	2/1/2013	1/31/2014	Year 1	
	\$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 Raymond J Grill PhD

RG Targeting inflammation in ALS using a dual COX/LOX inhibitor \$96,082.00 8/1/2012 7/31/2013 Year 2

Riluzole is the sole FDA-approved treatment for ALS. However, Riluzole treatment *Summary* enhances lifespan by only a few months. There is a clear need to identify novel interventions that are: 1) as, if not more, effective than Riluzole, and/or 2) able to enhance the efficacy of Riluzole when delivered in combination. Riluzole is a known substrate of the P-glycoprotein (Pgp), a component of the bloodbrain-barrier responsible for restricting access to the central nervous system of a wide range of biological substrates. Pgp levels are known to be increased by pro-inflammatory mediators such as prostaglandins and leukotrienes when tested both in vivo and in vitro. Milane and colleagues recently demonstrated that Pgp expression was dramatically elevated and bioavailability of Riluzole diminished in a mouse model of ALS that has exhibits high inflammatory activity. Licofelone is a next-generation anti-inflammatory drug in phase III trials for rheumatoid arthritis that inhibits both cyclooxygenase as well as 5-lipoxygenase; enzymes responsible for the production of prostaglandins and leukotrienes. We will determine whether Licofelone treatment can preserve function and enhance lifespan in the G93a mouse model of ALS. We will also determine whether Licofelone treatment with Riluzole enhances bioavailability of Riluzole and enhances efficacy compared to Riluzole treatment alone. Search terms: ALS, Licofelone, prostaglandins, leukotrienes, Riluzole, P-glycoprotein

Vasanthi Jayaraman PhD

RG	RNA Based Drugs for ALS				
	\$98,061.00	8/1/2012	7/31/2013	Year 2	
	\$98,061.00	8/1/2013	7/31/2014	Year 3	

Summary ALS is a neurodegenerative disease usually fatal within five years after diagnosis. This disease is characterized by progressive loss of upper and lower motor neurons, which causes the muscles under their control to weaken and die. An increase in the fraction of calcium permeable alphaamino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors has been observed in patients with

sporadic ALS, implicating these receptors in the pathalogical process of neurodegenration. The drugs currently available that target this class of proteins are not effective mainly because of their non-specificity associated side effects and due to their insolubility that leads to liver necrosis. We have successfully evolved RNA based ligands that are water soluble and act as inhibitors of the AMPA receptors and have shown that these ligands have neuroprotective properties in cell culture models. Here we propose to build on this excellent background and make these more selective to the subunit responsible for the increase in calcium permeability and also take it to the next phase of drug discovery by modifying these RNA based drugs to better their pharmacological properties such that they can be used in future pre-clinical investigations.

Vihang A Narkar Ph.D

RG Regulation of oxidative slow-twitch muscles by ERRgamma in DMD \$95,252.00 7/1/2012 6/30/2013 Year 3

Summary One emerging strategy to treat Duchenne muscular dystrophy is to increase aerobic muscles that have enhanced oxidative capacity and resistance to fatigue. Therefore, discovery of regulatory factors that increase aerobic muscles is of paramount importance in treating DMD. We have found that over-expression of ERRgamma by genetic engineering in mouse skeletal muscle activates genes that encode a highly aerobic and fatigue resistant muscle, suggesting that targeting this protein might be beneficial in muscular dystrophy. The current project is designed to investigate the extent to which ERRgamma rescues the pathology of DMD by increasing aerobic fatigue-resistant muscles. We plan to achieve this by genetically activating ERRgamma in the skeletal muscles of mdx mice, a rodent model of DMD, and measuring the ameliorative effects of the protein in restoring muscle architecture and performance. Our research has future implications in designing novel therapies to treat DMD by remodeling the muscle type.

Eric J Wagner Ph.D.

	- 0 -				
RG	Developing Dux4 3' End Formation Molecular Antagonists				
	\$93,542.00	8/1/2012	7/31/2013	Year 2	
	\$93.542.00	8/1/2013	7/31/2014	Year 3	

Summary Recent provocative data demonstrates that expression of the Double Homeobox 4 (Dux4) gene may be a significant determinant in the manifestation of Facioscapulohumeral Muscular Dystrophy (FSHD). Abnormal accumulation of Dux4 in FSHD patients is the result of changes in the DNA structure, most notably the placement of a flanking sequence allowing for the release of the Dux4 messenger RNA. This release of the Dux4 messenger RNA is an essential event resulting in the overproduction of Dux4 and ultimately the disease. The goals of this project are to understand the mechanism of Dux4 mRNA release and then design and test molecular tools aimed at antagonizing this event. We will first characterize the Dux4 flanking sequence to identify key DNA sequences required to allow for Dux4 accumulation. With this information in hand, we will then design minigenes capable of interfering with the Dux4 mRNA release in muscle cells. These results will form the basis to create chemically modified antagonistic DNA molecules designed to reduce the levels of Dux4. These molecular tools have shown considerable promise in clinical models of splicing diseases such as Spinal Motor Atrophy but our project will be the first to apply this technology to interfere with mRNA release. The long-term potential of this research will be to develop molecular therapeutic agents capable of specifically reducing the levels of Dux4 in FSHD patients.

UTAH

Salt Lake City - Sfida BioLogic, Inc.

John Paul Manfredi Ph.D.

RG	Evaluation in Mouse of Small Molecules to Treat Spinal Muscular Atrophy			
	\$83,576.00	2/1/2013	1/31/2014	Year 1
	\$78,419.00	2/1/2014	1/31/2015	Year 2

Summary We have identified novel small molecules that promote the growth of axons of motor neurons. We hypothesized that such "axonotrophic" effects of the chemicals, which we obtained using

rat spinal motor neurons, would have functional consequences. This hypothesis was supported in tests of the compounds on Drosophila mutants that exhibit locomotion defects. We further hypothesized that the chemicals would have positive effects in animal models of SMA, a disorder distinguished by degeneration of spinal motor neurons. In recent work financed by the Muscular Dystrophy Association, we found that the compounds dramatically suppress the aberrant morphology of motor neurons in a zebrafish model of SMA. We will now test the effects of the compounds in a mouse model of SMA. Importantly, previous tests of the compounds in healthy adult rats and mice showed that the chemicals are non-toxic, metabolically stable, capable of entering the central nervous system (CNS), and appropriately long-lived in the CNS and plasma. The compounds are therefore likely to be well-tolerated by the SMA mice, which we will confirm. Given the drug-like characteristics of the compounds, positive results in the SMA mice will nominate the compounds for clinical development. Moreover, positive results will have a significant impact on the strategies used to identify additional therapeutic candidates and the types of agents that are evaluated.

Salt Lake City - University of Utah Nicholas Johnson M.D.

RG	A Longitudinal Study of Disease Progression in Congenital Myotonic Dystrophy				
	\$125,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

VIRGINIA

Blacksburg - Virginia Polytechnic Institute and State University Hao Shi Ph.D.

DG	Role of MKP-5 in Duchenne Muscular Dystrophy				
	\$60,000.00	2/1/2012	1/31/2013	Year 2	
	\$60,000.00	2/1/2013	1/31/2014	Year 3	

Summary The mitogen-activated protein kinases (MAPKs) have been suggested to be critical for muscle regeneration and maintenance. However, the molecular details of the role the MAPKs in muscular dystrophy are unclear. The regulation of the MAPKs is of fundamental importance to our understanding of post-developmental skeletal muscle physiology and pathophysiology. Although much is known about how the MAPKs are activated little is known about how they are inactivated in skeletal muscle function. The MAPK phosphatases (MKPs) are a family of dual-specificity phosphatases that directly inactivate the MAPKs. We show that mice deficient for the MKP, MKP-5, exhibit improved adult muscle regeneration following injury. Mice lacking both dystrophin and MKP-5 have improved myopathy. We have found that satellite cells derived from MKP-5-deficient mice proliferate more rapidly and these mice show increased regenerative capacity. Therefore, we hypothesize that MKP-5

negatively regulates adult regenerative myogenesis. We will test this hypothesis by 1) characterizing the muscle phenotype in the MKP-5/dystrophin-deficient mice, 2) defining the physiological role of MKP-5 in muscle growth and development, 3) establish the molecular mechanism through which MKP-5 signals in skeletal muscle. These studies will identify novel pathways controlling skeletal muscle regeneration and may potentially uncover novel therapeutic targets for the treatment of Duchenne muscular dystrophy.

Charlottesville - The Rector and Visitors of the University of Virginia Mani S. Mahadevan M.D.

RG Characterizing RNA Foci in DM1

\$145,000.00 2/1/2012 1/31/2013 Year 2 \$145,000.00 2/1/2013 1/31/2014 Year 3

Summary Myotonic dystrophy type 1 (DM1) is the most common inherited neuromuscular disorder in adults and children. Common features of DM1 include myotonia, progressive skeletal muscle loss, cardiac conduction abnormalities, insulin resistance, cataracts and in severely affected children it results in developmental problems and mental retardation. DM1 is caused by a (CTG) triplet repeat expansion in the DMPK gene that results in nuclear entrapment of the mutant DMPK mRNA. These "RNA foci" are thought to lead to RNA toxicity by affecting the function of RNA binding proteins that interact with the "toxic" RNA. Over the years, many people have asked me, what is a RNA foci in DM1? What RNAs are there and what proteins are part of the complex? That is difficult to answer since there is no current method for purifying RNA foci, let alone purifying them intact with all their components. Currently, little is known about the composition of RNA foci in DM1 other than the fact that they contain mutant DMPK mRNA and MBNL1 co-localizes with the foci. Nothing is known about protein-protein interactions at RNA foci. FRET gives us an opportunity to take a novel approach at characterizing interactions in RNA foci, and to study RNA foci further and examine their role in the disease process. Furthermore, these assays will be used as important tools for identifying drugs and their mechanisms of action.

Mani S. Mahadevan M.D.

RG	Identification of small molecule modulators of myogenic defects in DM1					
	\$140,676.00	2/1/2012	1/31/2013	Year 1		
	\$140,676.00	2/1/2013	1/31/2014	Year 2		

The aim of this proposal is to identify potential therapies for myotonic dystrophy type 1 Summarv (DM1). A key component of this study is the collaboration between the Mahdevan lab and Novartis through the Genomics Institute of the Novartis Research Foundation (GNF) to identify compounds that correct myogenic defects in DM1. DM1 is one of the most common inherited neuromuscular disorders in children and adults. It is a degenerative disease that causes a wide range of symptoms including muscle weakness and wasting, involuntary clenching of hands and jaw, swallowing problems, eye problems, heart disorders, extreme fatigue and other difficulties. There is no drug therapy available. Current treatment is directed towards alleviating the muscle symptoms and managing disabilities. The muscle weakness in DM1 is associated with an inability to develop mature muscle cells from precursor cells in response to muscle breakdown. We have developed both a muscle cell line and the corresponding mouse model that recapitulates this. Our goal is to identify small molecule compounds through a high throughput screen (HTS) of 850,000 low molecular weight compounds that can be evaluated in the mouse model for proof-of-principle studies of the improvement of the generation of mature muscles in DM1. Compounds identified through this process can then be used as starting points for further development of a therapeutic to DM1.

WASHINGTON

Pullman - Washington State University Buel D. Rodgers Ph.D.

RIG Washington Center for Muscle Biology, Exercise Physiology Phenotyping Core \$71,171.00 11/1/2012 10/31/2013 Year 2

\$73,965.00 11/1/2013 10/31/2014 Year 3

Summary The newly established Washington Center for Muscle Biology (WCMB) includes almost 70 faculty labs and provides access to several core facilities, but not those for assessing exercise performance. This is problematic as a comprehensive assessment of exercise performance is an excellent means for evaluating new strategies for treating Duchene muscular dystrophy and many other myopathies. Thus, expanding resources for the WCMB, including those to assess exercise performance in mice, will help a large cadre of scientists with research and training interests in muscular dystrophy. Our long-term goal is to establish the WCMB as a premier muscle biology research unit in the country. The objective of this proposal is to establish an Exercise Physiology Phenotyping core with modular metabolic treadmills and digital running wheels. This equipment is particularly useful in assessing genetic models for muscular dystrophy and in evaluating gene and cell therapeutics in pre-clinical studies. Our justification is that this equipment will provide a necessary resource to the WCMB community and will further enhance muscular dystrophy research at several biomedical research institutions in the region. These efforts will not only assist center development, but will help foster intellectual exchange and technology transfer between academia and the state's thriving biotech industry.

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	8/1/2013	7/31/2014	Year 1	
	\$100,000.00	8/1/2014	7/31/2015	Year 2	
	\$100.000.00	8/1/2015	7/31/2016	Year 3	

Duchenne Muscular Dystrophy (DMD) is a fatal, X-linked muscular dystrophy affecting **Summary** 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 AAVmediated 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 - Seattle Institute for Biomedical and Clinical Research Brian Kraemer Ph.D.

RG	Modulating TDP-43 phosphorylation for motor neuron protection in ALS				
	\$105,519.00	2/1/2012	1/31/2013	Year 1	
	\$105,519.00	2/1/2013	1/31/2014	Year 2	
	\$105,519.00	2/1/2014	1/31/2015	Year 3	

Summary Lesions containing abnormal TDP-43 protein are present in affected brain and spinal cord nerve cells in amyotrophic lateral sclerosis (ALS) patients. Recently, mutations in the TDP-43 gene have been shown to cause inherited ALS in some families. How abnormalities in TDP-43 protein causes nerve cells to die in ALS remains poorly understood. However, addition of phosphates to TDP-43 at abnormal positions is the most consistent hallmark of ALS related nerve cell destruction seen upon post

mortem examination of the nervous systems of effected patients. Furthermore, previous published studies in our lab using C. elegans have demonstrated that the addition of phosphates by kinase enzymes is critical for TPD-43 to be toxic to nerve cells. Ongoing work in the lab has identified 14 kinases potentially involved in TDP-43 phosphate addition. Here we propose to test which kinases act directly on TDP-43 and then test whether or not drugs that block those kinases prevent TDP-43 phosphate addition. Previous drug development efforts blocking kinases have been successful for other diseases. Our goal is to find drugs that block TDP-43 phosphate addition in cell culture and then test them in mouse models of ALS with the ultimate objective of finding compounds suitable for therapeutic intervention.

Seattle - University of Washington Joseph A Beavo Ph.D.

RG	Proposal: Mechanism of sildenafil action in muscular dystrophy				
	\$137,500.00	2/1/2013	1/31/2014	Year 1	
	\$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.

Gregory Block Ph.D.

DG	Mechanism of Wnt signaling in FSHD					
	\$59,998.00	8/1/2013	7/31/2014	Year 1		
	\$59,998.00	8/1/2014	7/31/2015	Year 2		
	\$59,998.00	8/1/2015	7/31/2016	Year 3		

Summary Facioscapulohumeral Muscular Dystrophy (FSHD) is thought to be caused by inappropriate activation of the transcription factor, Double Homeobox Protein 4 (DUX4). We have developed a cellular assay for FSHD that recapitulates the key components of the disease in that primary myotubes from FSHD patients undergo cell death in a DUX4-dependent manner. The benefit of the model system is that we can measure DUX4 and related toxicity in the appropriate cell type without overexpressing the protein. Because the myotube death occurs by activation of the cells own DUX4, we are able to query pathways that regulate DUX4 expression with the hope of identifying factors that prevent DUX4 expression. We have scaled the model for high-throughput analysis, and have found that the Wnt/ß-catenin signaling pathway, a pathway known to be involved in skeletal muscle homeostasis, represses DUX4. In this project we will determine which components of canonical and non-canonical Wnt signaling pathways regulate DUX4 expression in order to expand potential therapeutic targets for the disease. We will determine whether Wnt signaling regulation of DUX4 is dependent on chromatin structure at D4Z4. Finally, we will validate whether drugs targeting components of the Wnt pathway can be used to reduce DUX4 levels.

Jeffrey S Chamberlain Ph.D.

RRG	AAV vectors for gene	AV vectors for gene therapy of DMD				
	\$328,628.00	2/1/2013	1/31/2014	Year 1		

Summary Duchenne muscular dystrophy (DMD) is a severe, x-linked recessive genetic disease. Affected children present with progressive muscle weakness, typically lose the ability to walk in their

teenage years, and often require respiratory and cardiac support in their twenties. There is no cure, and current therapies are only able to slow disease progression and provide postural, heart and breathing support. Gene therapy could be an ideal solution for this genetic disorder. Finding a way to deliver the genetic therapy to every muscle in the body, having the gene work normally, getting the therapy to last long term, and finding a way to reverse damage which has already occurred are all major components to curing this disease. Our group was the first to show that new genes can be delivered to all the muscles of an adult animal. Our previous studies show we can significantly stop disease progression and improve muscle function in dystrophic mice and dogs by delivery of a new micro-dystrophin gene carried in a delivery shuttle known as AAV. Importantly, AAV gene therapy should work in all patients and does not depend on which mutation an individual carries. This application is to finalize planning for a human clinical trial of the safety of AAV gene therapy, and to improve methods for treating limb muscles of large animal models. The studies are directly related to moving gene therapy for DMD/BMD into clinical trials.

Joel R. Chamberlain Ph.D.

RG	RNA interference-based treatment of FSHD modeled in mice				
	\$110,260.00	8/1/2012	7/31/2013	Year 1	
	\$110,260.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Martin K Childers Ph.D.

ACTG	Gene therapy in canine myotubular myopathy				
	\$122,123.00	4/1/2012	3/31/2013	Year 2	
	\$126,487.00	4/1/2013	3/31/2014	Year 3	

Summary Our goal is to bring to clinical trial a new treatment for patients with X-linked myotubular myopathy, a devastating inherited muscle disease caused by mutations in the gene encoding the muscle protein myotubularin. In patients, the disease results in profound muscular weakness, breathing failure and early death. Currently, there is no effective treatment available to patients with this disease. Our group tested a gene therapy in a mouse model of myotubular myopathy and found that this treatment restored strength in severely weak mouse muscles. We recently discovered dogs that harbor the same mutation in myotubularin, bred these dogs, and are now ready to test a gene therapy in dogs with myotubular myopathy. Due to the dog's large body size, similar genes, muscle physiology, and clinical course, our proposed dog trial represents a critical step towards first-inhuman studies.

Martin K Childers Ph.D.

RG	Dystrophin-deficient cardiomyocytes for high-thruput drug screening				
	\$160,000.00	8/1/2012	7/31/2013	Year 2	
	\$160,000.00	8/1/2013	7/31/2014	Year 3	

Summary Heart failure is a common and serious feature of Duchenne muscular dystrophy. The reason for this is because the heart muscle carries a genetic mutation that damages the normal operation of this all-important muscle. Our project will allow for the discovery of new drugs that might reverse, or prevent effects of the disease on the heart muscle in DMD patients. We will use two types of new technology to find potential new drugs. First, we will use a groundbreaking method called "cellular reprogramming". This method was first used to make stem cells out of skin cells from patients. In our project, we will first make stem cells from the skin cells of DMD patients, then we will use these stem

cells to form beating heart cells. These newly "reprogrammed" heart cells will contain the same genetic mutation found in the patient's own skin cells. Many thousands of reprogrammed cells can be generated to form identical heart cells, and these cells can be individually examined. This remarkable new technology will allow us to study how a genetic mutation affects the heart cells of a specific patient. The second method we will use is a drug discovery "platform" that can screen individual cells against thousands of drug compounds available for testing. By marrying these two incredible technologies, this project will allow for the first time, the ability to test new drugs directly on the heart cells from an individual patient without carrying any risk to the patient.

Deok-Ho Kim Ph.D.

RG	Functional Restorati	on of Dystrophic Muscle	using Bioengineered C	ell Patches
	\$130,000.00	2/1/2013	1/31/2014	Year 1
	\$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.

Leo Pallanck Ph.D.

RG	Influence of the Mite	ochondrial Quality Contr	rol System on Mitochon	drial Myopathy
	\$104 233 00	7/1/2012	6/30/2013	Vear 3

Summary Mitochondria play critical biological roles in muscle and nerve cells and mitochondrial DNA (mtDNA) mutations cause a number of debilitating mitochondrial encephalomyopathies. Because mtDNA is essential for proper mitochondrial function and there are numerous copies of mtDNA per cell, in many mitochondrial encephalomyopathies the mtDNA mutation coexists in cells with normal mtDNA, a condition known as heteroplasmy. The cellular ratio of mutated to normal mtDNA plays a critical pathological role in mitochondrial encephalomyopathies, but the mechanisms that influence this ratio are largely unknown. We, and others have recently found that evolutionarily conserved proteins known as PINK1 and Parkin promote the fragmentation and degradation of damaged mitochondria. From these findings, we hypothesize that PINK1, Parkin and other components of the mitochondrial quality control system play an important role in the heteroplasmic state by acting to selectively target mitochondria containing mtDNA mutations for degradation. To test this hypothesis, we propose to use the model system, Drosophila melanogaster, to examine the influence of genetic alterations of the mitochondrial quality control system on the frequency and pathology of deleterious mtDNA mutations. Given our current ignorance of the factors that influence the frequency of mtDNA mutations and the importance of this process to human health, our studies should have broad biological and medical significance.

Morayma Reyes M.D., Ph.D.

RG	Role of PDGF Receptor alpha signaling in DMD cardiac fibrosis				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

AUSTRALIA

Clayton - Monash University

Peter David Currie PhD

\$125	Small molecule scre	ening in a zebrafish mode	el of Duchenne Muscul	ar Dystrophy
	\$125,000.00	8/1/2012	7/31/2013	Year 2
	\$125,000.00	8/1/2013	7/31/2014	Year 3

Summary Duchenne and Becker Muscular Dystrophy (MD) are allelic muscle wasting conditions arising from mutations in the dystrophin (DMD) gene. While the current animal models of DMD have generated valuable insights to the pathological basis of the disease each has its limitations. The most commonly used model, the mdx mouse, lacks many aspects of the human DMD pathology, but does possess the ability to be genetically manipulated with relative ease. Other mammalian model systems, such as the dystrophin-deficient dog, do reflect the human condition more closely, but have other disadvantages that make them less valuable for evaluating therapeutic strategies. Thus, an animal model that possesses a highly penetrant dystrophic phenotype, that could be genetically manipulated, would be a valuable adjunct to existing models. To this end we have established zebrafish as a model system in which to determine the mechanistic basis of DMD pathology. We have isolated mutations in the zebrafish dystrophin gene and have determined that Dystrophin-deficient zebrafish accurately model the human condition. In this project we will utilize the advantages of the zebrafish system to undertake the first in vivo drug screen for small molecules that can inhibit the onset of dystrophic symptoms in an animal model of DMD

CONCORD - Anzac Health & Medical Research Foundation Garth A Nicholson M.D., PhD

RG	Determining the Pat	Determining the Pathogenic Effects of ATP7A Mutations in Distal Motor			
Neuropathy					
	\$140,000.00	2/1/2012	1/31/2013	Year 1	
	\$140,000.00	2/1/2013	1/31/2014	Year 2	
	\$140,000.00	2/1/2014	1/31/2015	Year 3	

Summary We have discovered mutations in the copper transport gene ATP7A that cause X-linked distal motor neuropathy (distal HMNX). The gene defect causes a slow but progressive degeneration of the ends of the long motor neurons which drive the limb muscles. The ATP7A protein is essential for human copper metabolism. It is involved with the delivery of copper for physiological processes as well as maintaining copper balance in humans. We have shown that the mutant ATP7A protein does not traffic properly in the presence of elevated copper. This project brings together diverse complementary skills from two laboratories with expertise in peripheral neuropathies and copper metabolism respectively. We will use cell and mouse models to determine the biological and cellular effects of the impaired ATP7A trafficking caused by distal HMNX mutations.

Crawley - The University of Western Australia Kristen Jean Nowak Ph.D.

RG	Discovering modifier genes for neuromuscular disease severity				
	\$166,399,00	8/1/2012	7/31/2013	Year 2	

Summary In many neuromuscular disorders, the same genetic defect, even in one family, can cause either mild or severe disease. e.g., some patients with recessive skeletal muscle actin disease are very severely affected, whilst other patients with the exact same genetic defect have a milder form. This is probably due to other genes that affect disease severity. If we can find these genes, we may be able to develop new treatments for skeletal muscle actin disease. We will use special mice which all die before 9 days after birth as models of severely affected patients with recessive skeletal muscle actin disease. We will breed these mice with various lines of unique "Gene Mine" mice, which have been especially created to find genes for complicated traits. These Gene Mine mice have great genetic diversity and their genes are well characterized. If any of the offspring created by breeding our severely affected actin

disease mice with the various Gene Mine mice live longer than 9 days, we can quickly determine which genetic factors from the Gene Mine mice are responsible. We previously showed that cardiac actin could rescue these severely affected mice, enabling them to live until old age. Therefore there's great promise that this study will identify modifying genes that activate cardiac actin, or indeed another gene rescuing the mice through another mechanism. These genes will become the focus of future studies determining how to utilize them to formulate treatments for patients.

Steve D Wilton Ph.D

RG Preclinical assessment of splice switching oligomers

\$122,700.00 7/1/2012 6/30/2013 Year 3

Summary We have developed genetic bandaids (oligomers) to induce exon skipping to by-pass DMD-causing mutations. From a concept first demonstrated in vitro, then in animal models and first proof of concept studies in man, clinical trials are underway to evaluate two bandaid types (20Me and PMO). Another bandaid type (MOE) has a proven safety record, with clinical application for more than 12 months in over 1000 individuals. MOE bandaids appear well suited for exon skipping, but until now these oligomers have not been available for study. We will undertake detailed pre-clinical studies in animal models to assess their suitability for exon skipping. We have developed scores of AOs to by-pass other amenable dystrophin mutations. An often-cited and valid concern regards potential off-target effects, due to either the bandaid chemistry or non-specific binding. Could splice switching dystrophin oligomers anneal to similar RNA sequences and subsequently influence their expression? This project will detect potential cross-reactions between our optimized dystrophin bandaids and other gene sequences. Experiments will confirm predicted expression changes as well as variation associated with the nature of the bandaid types used. If these compounds are shown to be very specific, this would confer greater confidence that non-specific effects will be minor. If some bandaids caused adverse events, this data could be used to develop strategies to minimize or by-pass the problem.

Murdoch - Murdoch University Steve D Wilton Ph.D

RG	Oligomer design & v	alidation for DMD: quan	tum improvements in 6	exon skipping
	\$100,000.00	8/1/2013	7/31/2014	Year 1
	\$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 - Murdoch Childrens Research Institute Shireen R. Lamande Ph.D.

RG Identifying new genes for the collagen VI-related muscular dystrophies.

\$124,861.00 2/1/2012 1/31/2013 Year 2

Summary Mutations in the extracellular matrix protein collagen VI underlie two muscular dystrophies, Bethlem myopathy and Ullrich congenital muscular dystrophy (UCMD). One of our research arms has focused on identifying collagen VI mutations in these patients and understanding how and why the mutations cause muscle disease. To date we have screened 100 patients with Bethlem myopathy and UCMD and have found mutations in 62. The remaining 38 patients do not have collagen VI mutations. Other laboratories have also found that 30-40% of Bethlem and UCMD patients do not have collagen VI mutations and this indicates that mutations in other, as yet unidentified, genes also underlie these disorders. To identify new muscular dystrophy candidate genes in these patients we have screened out 38 patients who do not have collagen VI mutations for copy number changes in their genomic DNA using microarrays. We have identified copy number variations, mostly deletions, in eleven patients. In this project we will examine the genes in the altered regions, using bioinformatics, sequencing, and protein analyses, and identify new muscular dystrophy candidate genes. This will improve diagnosis for patients and families and increase our understanding of muscle biology and pathology.

Joseph Sarsero Ph.D.

RG Development of an improved GAA repeat expansion mouse model of Friedreich ataxia \$138,750.00 2/1/2012 1/31/2013 Year 2

\$138,750.00 2/1/2012 1/31/2013 Year 2 \$138,750.00 2/1/2013 1/31/2014 Year 3

Summary The genetic defect that causes Friedreich's ataxia (FRDA) results in reduced levels of an essential protein termed frataxin in all cells of the body. Prior to evaluating new therapies in patients it is important that they be tested in appropriate biological models of the disease. Animal models that are generated by the 'knockout' of specific genes often manifest the main symptoms of the corresponding human disorder, however such models rarely recapitulate the precise molecular cause that underlies human disease. Accurate 'humanized' mouse models of disease are designed to contain an entire human gene of interest and harbor the specific disease-causing mutation as found in patients. Such mice do not only manifest the main symptoms of a disorder, but also provide the correct underlying molecular cause of the disease. We will utilize our expertise in handling the gene responsible for FRDA, and our current preliminary mouse models of FRDA, to generate an improved humanized FRDA mouse model that more accurately reflects disease symptoms and the underlying molecular cause of the disorder. This will be an important resource for the study of the pathophysiology of the disease and for the evaluation of novel therapeutic interventions.

Margaret Rosemary Zacharin F.R.A.C.P

RG Clinical trial of Zoledronic acid in children and adolescents with Duchenne(DMD) \$129,850.00 7/31/2012 7/31/2013 Year 2 \$.00 8/1/2013 12/31/2013 Year 3

Chronic, progressive immobilization is an inevitable consequence of Duchenne muscular Summary dystrophy, a genetically determined male limited muscle disorder, with early childhood onset of symptoms. Corticosteroids have been shown to assist in keeping children with DMD more mobile for longer, without need for a wheelchair. They are now used in almost all DMD patients at chronic dose levels known to result in inevitable, progressive bone loss and increased fracture risk, both in long bones and vertebrae. The incidence of both upper and lower extremity long bone fracture in boys with DMD is between 21 and 44%, with a peak incidence in late childhood, most often resulting from minimal or no trauma and associated with significant pain and disability. Up to 20-50% of boys ambulant prior to fracture, lose ability to walk thereafter. Bisphosphonates are a class of drug taken into and bound to bone, reducing rate of bone turnover. They alter the course of corticosteroid induced bone loss and largely prevent this complication in adults. We propose to conduct a randomized trial of zoledronic acid (a bisphosphonate) over 12 months, in boys with DMD, aiming to demonstrate that it is superior to calcium and vitamin D, to improve bone density, bone turnover and vertebral shape. This study will then be used to power a larger study to assess fracture risk reduction in this group, in turn providing evidence for a new and successful intervention to improve health and quality of life in affected boy

Parkville - The University of Melbourne Gordon Stuart Lynch Ph.D.

RG Modulating IGF:IGFBP signaling to improve muscle function in muscular dystrophy \$125,000.00 7/1/2012 6/30/2013 Year 3

Muscle wasting and weakness are major symptoms of many muscular disorders, Summary 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, alternative therapies be developed, and research directed to preserving muscle tissue, enhancing muscle regeneration, and promoting muscle growth. We have demonstrated the exciting potential of growth factors like IGF-I for improving muscle function in mouse models of muscular dystrophy. The actions of IGF-I are strongly modulated by a family of IGF binding proteins (IGFBPs) that bind IGF-I. There is a major lack of understanding about the roles of IGFBPs in muscle, particularly in the pathophysiology of muscular dystrophy. There is evidence that one IGFBP in particular, IGFBP-2, plays a critical role in modulating muscle growth and our preliminary data shows that it may play a key role in the cycles of fiber damage and repair that are implicated in the etiology and progression of DMD. This project will examine the role of the IGFBPs, especially IGFBP-2, on the pathophysiology of muscular dystrophy in the mdx and dko mouse models of DMD. Utilizing transgenic mice that overexpress or lack IGFBP-2, we will determine whether IGFBP-2 aggravates or improves the dystrophic pathology. This project will generate entirely novel information about IGFBPs and IGF signaling in muscular dystrophy.

Gordon Stuart Lynch Ph.D.

RG	Therapeutic potential of heat shock protein 72 induction in muscular dystrophy				
	\$135,503.00	2/1/2013	1/31/2014	Year 1	
	\$135,003.00	2/1/2014	1/31/2015	Year 2	
	\$135,003.00	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, NSW - The University of Sydney Joshua Burns Ph.D

HCT	Strength training for children with Charcot-Marie-Tooth disease: Help or Harm?				
	\$51,161.09	10/2/2012	2/28/2013	Year 1	
	\$102,322.00	3/1/2013	2/28/2014	Year 2	
	\$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.

Des Richardson Ph.D., D.Sc.

RG	Development of Iror	n Complexes for the Trea	• • •	
	\$150,000.00	8/1/2012	7/31/2013	Year 2
	\$150,000.00	8/1/2013	7/31/2014	Year 3

Summary Our MDA funded studies within the last 3 years have led to important advances in understanding how Friedreich's ataxia leads to neuro-degenerative and cardio-degenerative problems. Specifically, we have discovered the molecular mechanisms that lead to mitochondrial iron- loading which is highly toxic. The current studies are a logical and novel extension of that work, and will lead to further understanding of not only the disease, but also the development of new treatments that take advantage of the knowledge discovered by our previous studies on the pathogenesis of this condition.

BELGIUM

Gent - Flanders Institute for Biotechnology and University of Antwerp Albena Jordanova PhD

RG	Identification of molecular	players and drug targe	ets for CMT neuropathio	es
	\$94,210.00	7/1/2012	6/30/2013	Year 3

Summary Dominant intermediate Charcot-Marie-Tooth disease type C (DI-CMTC) is a recently defined CMT entity, characterized by slowly progressive neuropathy, intermediate nerve conduction velocities along peripheral nerves and histological evidence of both axonal and Schwann cell involvement. We were the first to describe this genetic entity and demonstrated that it is caused by different mutations in the gene coding for tyrosyl-tRNA syntethase. This project is focused on the identification of molecular players and potential drug targets for this particular subtype of CMT. We will perform a screen for genetic modifiers of neurodegenerative phenotypes present in a Drosophila model for DI-CMTC. The genes will be selected based on their reported abilities to interact with drug-like compounds. In this way we will be able to gain original information on DI-CMTC pathomechanisms and to translate it into a rational and reliable drug discovery program. The knowledge gained will be relevant also to other inherited and acquired neuropathies.

CANADA ONTARIO

Ottawa - Children's Hospital of Eastern Ontario Research Institute Inc Robert Korneluk Ph.D

RG	Role of cIAP1 and cIAP2 in myogenesis and muscular dystrophy				
	\$141,984.00	2/1/2012	1/31/2013	Year 1	
	\$141,984.00	2/1/2013	1/31/2014	Year 2	
	\$141,984.00	2/1/2014	1/31/2015	Year 3	

Summary The Nuclear Factor kappaB (NFkB) signalling pathway is critical for normal skeletal muscle function, and in promoting recovery in response to muscle injury. However, there is strong evidence that NFkB signalling is also involved in the pathology associated with various muscle diseases, such as Duchenne Muscular Dystrophy (DMD). We have recently found that the cellular inhibitor of apoptosis 1 and 2 (cIAP1/2) proteins, which were initially identified by our research group, are required for various aspects of NFkB signalling in skeletal muscle. In preliminary studies, we have discovered that the loss of cIAP1 expression in muscle in cultured cells and in mice leads to perturbations in NFkB signalling pathways that improve muscle function and recovery. Most strikingly, when the mdx mouse model of DMD is bred with a mouse deficient in cIAP1 expression, the progeny mice show improvements in muscle pathology. This discovery has raised the exciting possibility that cIAP1 may be a potential therapeutic target for various muscle diseases that rely on NFkB signalling. We propose to elucidate the roles and mechanism of action of cIAP1 and cIAP2 in NFkB signalling in skeletal muscle,

and evaluate their contribution to the pathology of DMD. Moreover, we have in hand several drugs that specifically target cIAP1/2 for destruction (currently in clinical trials for cancer) and will evaluate the therapeutic potential of these drugs in the treatment of muscular dystrophy.

Ottawa - Ottawa Hospital Research Institute Lynn Megeney Ph.D.

RG	Caspase 3 Limits the Renewal of Activated Satellite Cells				
	\$100,000.00	8/1/2013	7/31/2014	Year 1	
	\$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.

Michael A. Rudnicki PhD

RG	Molecular Regulation of Satellite Cell Function				
	\$125,000.00	8/1/2012	7/31/2013	Year 2	
	\$125,000.00	8/1/2013	7/31/2014	Year 3	

Summary Muscle satellite cells are required for the growth and repair of skeletal muscle. Our laboratory identified a subset of muscle satellite cells that function as "satellite stem cells" that are capable of giving rise to committed "satellite myogenic cells" and of repopulating the satellite cell niche following transplantation. Recently, we discovered that a secreted protein called Wnt7a stimulates the division of satellite stem cells and also directly stimulates the growth of muscle fibers. Notably, we found that introduction of Wnt7a into normal and dystrophic muscle results in enhanced contraction strength of the tissue. In addition, we have found that the function of satellite stem cells is compromised in mdx mice, a mouse model of Duchenne Muscular Dystrophy (DMD), suggesting that dystrophin is required for the appropriate regulation of satellite stem cell function. In this application we propose a series of experiments to characterize the nature of the muscle stem cell defect in mdx mice. We will investigate the cell mechanism through which Wnt7a treatment induces an increase of satellite stem cell numbers and repair of dystrophin-deficient skeletal muscle. Finally, we will conduct experiments using mdx mice to investigate the utility of Wnt7a as a drug for the treatment of DMD.

Ottawa - The University of Ottawa Ilona Skerjanc Ph.D.

RG	Enhanced muscle repair with human embryonic stem cells				
	\$140,604.00	2/1/2012	1/31/2013	Year 1	
	\$139,883.00	2/1/2013	1/31/2014	Year 2	
	\$.00	2/1/2014	1/31/2015	Year 3	

Summary Cell therapies to reverse muscle atrophy and to strengthen skeletal muscle would greatly enhance and extend the lives of patients with dystrophic diseases, including muscular dystrophy. Several cell sources for therapy are currently under study by others, including satellite and mesenchymal stem cells. However, difficulties with these approaches include the requirement for invasive procedures, the availability of suitable donors, and a limited long-term proliferation potential. With funding from MDA, we have recently shown that human embryonic stem (hES) cells can differentiate into skeletal muscle via progenitor and myoblast stages. hES cells could provide an unlimited amount of skeletal

muscle progenitors, with enhanced in vivo potential. Previous work has reported the transplantation of hES-derived myoblasts into mice, indicating the promise of this approach for future therapeutic applications. However, the ability of these cells to contribute to the satellite cell niche and to enhance skeletal muscle function was not assessed. To this end, we will isolate skeletal muscle progenitors from hES cells and examine their ability to engraft into skeletal muscle in mdx mice, assessed by their contribution to the satellite cell niche, and enhancement of muscle function. The overall goal is to provide a method of hES cell differentiation and enrichment that will generate human myoblasts/progenitors for long-term engraftment and future therapeutic applications.

Ottawa - University of Ottawa Bernard Jasmin PhD

RG Impact of exercise mimetics on the dystrophic pathology in the mdx mouse \$120,000.00 7/1/2012 6/30/2013 Year 3

Summary One therapeutic strategy for Duchenne muscular dystrophy involves utilizing a protein normally expressed in dystrophic muscle which, 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 A because it is a cytoskeletal protein that displays a high degree of sequence identity with dystrophin. Additionally, muscle fibers from DMD patients express utrophin A endogenously. Therefore, studies aimed at deciphering the mechanisms involved in controlling utrophin A expression in skeletal muscle are important as they pave the way for target identification and rational design of specific pharmacological interventions focused on increasing the endogenous expression of utrophin A all along the sarcolemma of dystrophic muscle fibers.

Vladimir Ljubicic Ph.D.

DG	Dissecting the mechanisms underlying the benefits of novel therapeutics for DMD					
	\$60,000.00	2/1/2012	1/31/2013	Year 1		
	\$60,000.00	2/1/2013	1/31/2014	Year 2		
	\$60,000.00	2/1/2014	1/31/2015	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 slowtwitch 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.

Aymeric Ravel-Chapuis Ph.D.

DG Role of the RNA-binding protein Staufen1 in Myotonic Dystrophy type1 \$59,950.00 7/1/2012 6/30/2013 Year 3

Summary Myotonic Dystrophy type 1 (DM1) affects 1/8000 individuals worldwide and up to 1/500 in certain regions. The disease affects skeletal muscles, which become weak, painful and do not properly relax following contraction. It also affects other organs such as heart, eyes, nervous system, and endocrinal system. It is a genetic disorder caused by a mutation, a repetition of CTG trinucleotides, in the DMPK gene. The pathological RNA expressed from this gene is blocked into the nucleus of the cell where it aggregates. It becomes toxic to the cell because it sequesters proteins, preventing them from

assuming their normal functions and thereby causing the many symptoms characteristic of this disease. The current proposal is designed to examine the role of one such protein, called Staufen. Our work shows that Staufen interacts with the DMPK RNA, and that the modulation of Staufen levels in DM1 cells can affect mutant RNA accumulation in the nucleus and revert some features observed in the disease. The identification of such a protein and the elucidation of its functions in skeletal muscle is important since these studies may lead to the development of new therapeutic strategies for treating DM1.

Nadine Wiper-Bergeron Ph.D.

RG Improving myoblast transplantation outcomes by modulating C/EBPbeta expression.

\$105,165.00	2/1/2012	1/31/2013	Year 1
\$103,604.00	2/1/2013	1/31/2014	Year 2
\$103,653.00	2/1/2014	1/31/2015	Year 3

Summary One way to cure muscular dystrophies is to use stem cells to repair damaged muscle. These cells can help the damaged muscle become healthy, reversing the muscle mass loss and weakness. However, so far, this approach has not been very successful because in addition to repair we need some of the stem cells to live in the muscle all the time, to make more cells that can help with repair. My lab has discovered a protein called C/EBPbeta which helps stem cells in the muscle. We believe that if we treat donor muscle stem cells with a special drug called IBMX, we can make the transplant work better by not only repairing the muscle but also creating a population of healthy stem cells within the muscle. Over time, this new way to transplant cells will help patients make healthier muscle and will improve their quality of life.

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				
	\$81,100.00	8/1/2012	7/31/2013	Year 1	
	\$75,100.00	8/1/2013	7/31/2014	Year 2	
	\$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	8/1/2012	7/31/2013	Year 1	
	\$119,414.00	8/1/2013	7/31/2014	Year 2	
	\$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				
	\$119,322.00	2/1/2013	1/31/2014	Year 1	
	\$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.

Josephine Nalbantoglu Ph.D.

RG	Artificial zinc fingers targeting the human utrophin promoter			
	\$104.390.00	7/1/2012	6/30/2013	Year 3

Summary In Duchenne Muscular Dystrophy (DMD), repeated cycles of muscle fiber destruction lead to progressive paralysis and death. The basic cause of this is the lack of an essential protein, dystrophin, at the surface membrane of muscle fibers due to a mutation of a very large gene on the X chromosome. A promising approach to the therapy of DMD is the transfer into muscle fibers of a normal dystrophin gene by viral and non-viral vectors. Another molecular approach to DMD therapy is a substantial increase of an analogue of dystrophin, utrophin, so that it is expressed not only at its normal site of the neuromuscular junction but throughout the sarcolemma. Several pieces of evidence in mouse and dog models of DMD indicate that a substantial increase of the amount of the extrasynaptic utrophin will mitigate or eliminate muscle fiber damage caused by dystrophin deficiency. We have previously published that artificial transcription factors that target the promoter of mouse utrophin can also result in increased levels of utrophin promoter and mitigate muscle fiber damage. In this proposal, we will use the same approach to design artificial transcription factors which target the human utrophin promoter. It is hoped that these may eventually be used as part of therapy for Duchenne muscular dystrophy.

Montréal - Institut de recherches cliniques de Montréal (Clinical Research Institute of Montreal) Benoit Coulombe Ph.D.

RG	Regulation of the inclusion	n body myositis-asso	ciated protein VCP by methylatior	
	\$125.689.00	2/1/2013	1/31/2014	Year 1

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

Tissue degeneration is a hallmark of many muscular and neurological diseases. In many Summary 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.

Quebec - CHUL Research Center Jasna Kriz Ph.D.

RG	Live Imaging of ALS Pathogenesis and Therapeutic Efficacy				
	\$148,362.00	8/1/2012	7/31/2013	Year 2	
	\$148.362.00	8/1/2013	7/31/2014	Year 3	

Summary The pathological events that precipitate the clinical onset of amyotrophic lateral sclerosis are not yet well understood. To address these important issues we recently developed novel ALS mouse models in which we can visualize early pathological changes from living animals using in vivo imaging technologies. Our objective is to study early pathological signals (changes) from live animals and use this knowledge to create novel therapeutic strategies for ALS and possibly other neuromuscular disorders.

Québec - Université Laval François Berthod Ph.D.

RG	Development of a human tissue-engineered model of the spinal cord to study ALS			
	\$115,698.00	2/1/2012	1/31/2013	Year 2
	\$115,698.00	2/1/2013	1/31/2014	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is due to preferential degeneration of motor neurons in the spinal cord and brain that control muscle movements. Our objective is to develop a three dimensional in vitro model of the spinal cord to study in vitro the interactions of different cell types [motor neurons (MN), astrocytes, microglia, Schwann cells] in a highly physiological environment. In addition, our aim is to develop this model using human neural cells obtained from post-mortem ALS patient tissues to mimic the human disease in vitro. We have previously shown that human mature neurons can be differentiated from a population of multipotent stem cells isolated from human skin. We will develop a human tissue-engineered spinal cord model (TESC) to mimic motor neuron degeneration in vitro. It will permit the study of various combinations of cells differentiated from ALS patient or normal subjects, in order to determine which specific conditions induce or participate in MN death and the mechanism responsible. This work will certainly improve our understanding of the causes and progression of sporadic ALS (90% of ALS patients without familial history of ALS).

Jean-Pierre Julien Ph.D.

RG	Chromogranin variants as r	risk factor and modifier	on disease onset for A	۱LS
	\$115,000.00	7/1/2012	6/30/2013	Year 3

Summary Recently we discovered the existence of a common variant of chromogranin B gene (CHGB) that acts as susceptibility factor and modifier of disease onset in ALS. We will conduct transfection experiments in cultured neuronal cells and will generate transgenic mouse models to elucidate the pathogenic mechanisms by which a chromogranin B variant gene may act as risk factor in ALS. Our hypothesis is that this chromogranin variant can contribute to increase the vulnerability of motor neurons by causing a dysfunction of the secretory pathway and alterations of ER-Golgi homeostasis. Furthermore, we will study the interaction of the CHGB variant with mutant superoxide dismutase (SOD1) and determine whether expression of the CHGB variant will affect disease onset and duration in mice expressing mutant SOD1G37R.

CHILE Santiago - Institute of Biomedical Sciences, Faculty of medicine, University of Chile Claudio A Hetz Ph.D

RG	Role of ER stress in SOD1 wild-type misfolding: A model for sporadic ALS?				
	\$72,500.00	2/1/2012	1/30/2013	Year 1	
	\$72,500.00	2/1/2013	1/30/2014	Year 2	
	\$72,500.00	2/1/2014	1/30/2015	Year 3	

Summary Amyotrophic lateral sclerosis (ALS) is a progressive and deadly adult-onset motoneuron disease. The majority of ALS patients lacks a defined hereditary genetic component and is considered sporadic (sALS). The primary mechanism responsible for the progressive motoneuron loss in ALS remains unknown. Clues have been obtained from families with familial ALS, which are accompanied by alterations in the folding of important proteins called superoxide dismutase (SOD1) and TAR-DNA binding protein 43 (TDP43). Remarkably, these proteins are also altered in sALS cases, however, the pathological events underlying the misfolding of wild-type SOD1, and TDP43 are completely unknown. Interestingly, perturbations of the protein folding functions performed at a subcellular organelle called endoplasmic reticulum (ER) occur in sALS and have been suggested to determine the neurotoxicity of ALS-linked mutant SOD1. We have obtained preliminary data supporting the involvement of ER stress and specific ER folding mediators (foldases) in the pathological misfolding of wild-type SOD1, resembling what is observed in sALS-derived tissue. Here we will define the impact of specific foldases to motoneuron dysfunction in ALS. Using animal models of the disease and cell culture experiments we plan to assess possible therapeutic benefits of manipulating ER foldases in ALS. This work may lead to the design of novel therapeutic strategies to treat this fatal neuromuscular disease.

COSTA RICA

San José - Universidad de Costa Rica Fernando Morales Ph.D

RG	Myotonic dystrophy: understanding its somatic mutational dynamic and modifiers			
	\$125,000.00	8/1/2012	7/31/2013	Year 2
	\$116,210.00	8/1/2013	7/31/2014	Year 3

Summary Myotonic dystrophy (DM) is caused by a mutation that makes the DM gene expand. Due to the highly clinical variability of the DM, it has been difficult to establish a precise relationship between the size of the mutation and the age of onset and disease severity. It is known that the size of the mutation increases through life and when it is transmitted through generations. However, it is unknown how the mutation occurs, the way the mutation increases, if the rate of change correlates with the age of onset and progression of the disease, and other genetic factors that might be involved in the mutational mechanism. The final outcome of the age of onset and the clinical variability seems to be due to a combination of yet unidentified genetic and environmental factors. Thus, this project is aimed to analyse how the DM mutation changes over time and how it relates with the clinical picture of the patients; the major modifiers of the mutation size variability and change, but also genetic modifiers of the mutation that might explain individual's specific variation. Those modifiers that show a relationship with the mutation could be used as therapeutic targets in order to delay onset and progression of the

disease. This project could generate more truthful genetic data for DM that could provide more accurate prognostic information to the DM patients.

CYPRUS

Nicosia - CING - The Cyprus Institute of Neurology and Genetics Kleopas A. Kleopa M.D.

RG	Developing gene therapy for inherited neuropathy				
	\$91,663.00	8/1/2013	7/31/2014	Year 1	
	\$93,287.00	8/1/2014	7/31/2015	Year 2	
	\$95,995.00	8/1/2015	7/31/2016	Year 3	

Our aim is to develop and test a novel gene therapy for a common inherited *Summary* 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	Identification of novel genes mutated in centronuclear myopathies				
	\$119,750.00	2/1/2012	1/31/2013	Year 2	
	\$101,750.00	2/1/2013	1/31/2014	Year 3	

Summary Centronuclear myopathies are rare and severe myopathies linking muscle weakness and atrophy to abnormal structure of the muscle fibers. Several forms exist with onset at birth, infancy or adulthood and can vary in severity. Most patients suffer from severe forms that lead to respiratory difficulties and necessary ventilation assistance. These conditions could be life threatening and impose a burden to families and society, as they are often progressive and accompanied with other medical complications. There is no specific therapeutic approaches to date. Several responsible genes have been previously found but do not account for all patients by far. Thus additional implicated genes remain to be identified. This is important as gene identification allows access to molecular diagnosis (to confirm the clinical diagnosis), genetic counseling in families and further identification of the pathological mechanisms leading to these diseases. Moreover, the knowledge of the implicated genes is often compulsory to access therapeutic trials and treatments. We aim to identify novel genes implicated in centronuclear myopathies by high throughput approaches. These genes will also represent novel therapeutic and drug targets.

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	2/1/2013	1/31/2014	Year 1
\$115,011.00	2/1/2014	1/31/2015	Year 2
\$115,011.00	2/1/2015	1/31/2016	Year 3

The low-density lipoprotein receptor-related protein 4 (LRP4) presents a novel Summary 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	8/1/2012	7/31/2013	Year 1	
	\$100,003.00	8/1/2013	7/31/2014	Year 2	
	\$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.

Tel Aviv - Tel - Aviv University Daniel Offen Ph.D.

RG Myogenic cells – possible application in autologous cell and gene therapy of ALS \$119,900.00 7/1/2012 6/30/2013 Year 3

Summary The cause of motor neuron death in ALS is not fully understood and even the exact cell types involved in the disease is unknown. Neurotrophic factors are advantageous agents that have been reported as beneficial in rodent models of ALS. However, up to date, none of the tested factors has lived up to expectations, probably because of rapid degradation of the factors. The present study aims to further investigate this direction using a better delivery system of these beneficial factors. Transplanted muscle progenitor cells stably integrate in the damaged muscle tissue. In this project, muscle progenitor cells, which have been engineered to express combinations of various neurotrophic factors, will be injected into the muscles of ALS affected mice and the effect on the progression of the disease will be

followed. Similar experiments will be performed in ALS mice injected with muscle progenitor cells isolated from adult human muscle biopsies. This study is likely to contribute to a better understanding of ALS and may lead to a novel autologous cell/gene therapy approach

ITALY

Genova - Fondazione Istituto Italiano di Tecnologia Maria Pennuto Ph.D.

RG PKA signaling in SBMA pathogenesis

\$110,000.00 8/1/2012 7/31/2013 Year 2 \$110,000.00 8/1/2013 7/31/2014 Year 3

Summary The mutation responsible for spinal and bulbar muscular atrophy (SBMA), also called Kennedy's disease, is in the gene expressing the androgen receptor. We will test the hypothesis that activation of a protein, named protein kinase A, is beneficial because it reduces the accumulation of mutant androgen receptor. We will explore the mechanism through which activation of protein kinase A reduces the toxicity of mutant protein. Moreover, we will identify agents that can activate the kinase and in so doing reduce the toxicity of mutant protein. We have previously identified another kinase to protect SBMA skeletal muscle. Here, we will investigate the effect of protein kinase A to protect SBMA spinal cord.

Rome - University of Rome Tor Vergata

Claudio Sette PhD in Medical Embryology

RG Role of Sam68 in the regulation of SMN2 alternative splicing in SMA cells \$73,000.00 2/1/2012 1/31/2013 Year 2 \$73,000.00 2/1/2013 1/31/2014 Year 3

Human genes contain introns that need to be excised from the mRNA through a process **Summary** named splicing. The regulation of splicing is orchestrated by numerous proteins, RNAs and sequence elements located in the genes that compose the so called "splicing code". Up to 50% of disease-causing genetic mutations are thought to affect the splicing code without altering the open reading frames of the proteins encoded by the gene. One of the best characterized genetic diseases caused by such mutations is Spinal Muscular Atrophy (SMA), an autosomal recessive neuromuscular disorder representing the primary genetic cause of infant mortality. SMA is caused by inactivating mutations in SMN1, which encodes the Survival Motor Neuron protein (SMN). Although SMA patients retain the almost identical SMN2 gene, a single nucleotide change in SMN2 causes the exclusion of exon 7 from the mRNA and the production of an unstable protein. Hence, regulation of SMN2 splicing represents a valuable therapeutic approach for the correction of this disease-causing genetic defect. We have identified Sam68 as a novel splicing factor causing exon 7 skipping in SMN2 and have shown that inhibition of its function in SMA fibroblasts restores exon 7 inclusion and SMN expression (Pedrotti et al., EMBO J 2010). This project aims at further elucidating the function of Sam68 in neuronal cells and at understanding whether regulation of its activity can rescue SMN function in SMA neuronal stem cells and in SMA animal models.

NETHERLANDS

Leiden - LUMC

Jasprina N. Noordermeer Ph.D.

RG Elucidating the Synaptic Roles of Dystrophin

Summary The best understood role of Dystrophin, the protein absent in Duchenne Muscular Dystrophy (DMD) patients, is to stabilize the muscle. It is also present at synapses, the sites where neurons contact each other or muscles. Fully a third of boys with DMD have mental disabilities indicating that Dystrophin plays critical roles at brain synapses. Clinical studies have shown that these

cognitive impairments further reduce the quality and length of their lives. Ultimately, therefore, treatments for DMD must reverse both muscle wasting and the cognitive deficits but how the lack of Dystrophin results in synaptic defects is unclear. Dystrophin is highly conserved in evolution so findings made on Dystrophin in animals can reveal its roles in humans. Consequently, we study its roles at fruit fly model synapses using powerful genetic approaches. We found that lack of Dystrophin results in abnormal function of the neuron-muscle synapse and have uncovered a new muscle signaling pathway involved in this effect. We and others have also shown that fly and mouse synapses in the Dystrophin-deficient brain similarly malfunction, indicating that what we have learned from the fly neuron-muscle model translates to the brain. Here, we will identify new members of this pathway and determine whether it also acts at brain synapses. The human counterparts of the proteins that we find will serve as potential therapeutic targets for the treatment of DMD-associated mental disabilities.

Silvere Maria van der Maarel Ph.D.

RG	Molecular pathophysiology of FSHD2				
	\$131,560.00	2/1/2012	1/30/2013	Year 1	
	\$124,910.00	2/1/2013	1/30/2014	Year 2	

Summary A significant proportion of patients with FSHD show no contraction of the D4Z4 repeat on chromosome 4 – the genetic hallmark of FSHD – but show a relaxation of the D4Z4 repeat structure and expression of the DUX4 retrogene typically observed in patients with contraction of D4Z4. Based on our studies of families with this so-called FSHD2 we hypothesize that there is a genetic basis for this condition: these patients likely carry somewhere in their genome a genetic variant that causes the relaxation of the D4Z4 repeat and the expression of DUX4 in their muscle. In this proposal we will a) design and validate a reliable diagnostic technique for FSHD2 to be implemented in the diagnostic laboratories. Currently there is no diagnosis available for FSHD2; b) identify the genetic defect that underlies FSHD2. This will further improve the diagnostic possibilities for FSHD2 and provide mechanistic insight into its pathophysiology; and c) further delineate the epigenetic commonalties and differences of the D4Z4 locus in FSHD1 and FSHD2 to generate a chromatin map of D4Z4. We expect that this study will advance our knowledge of the disease mechanism of FSHD and deliver a reliable diagnostic procedure for FSHD2.

SINGAPORE

Singapore - National University of Singapore Chi Wai Lee Ph.D.

DG	The Mechanism of Postsynaptic AChR Endocytosis in Myasthenia Gravis
Pathogenesis	

U				
	\$60,000.00	2/1/2012	1/31/2013	Year 2
	\$60,000,00	2/1/2013	1/31/2014	Year 3

Summary At mature neuromuscular junctions (NMJs), acetylcholine receptors (AChRs) are highly concentrated on the postsynaptic membrane for effective reception of neurotransmitters released from the presynaptic nerve terminal. In 80-90% of patients with generalized myasthenia gravis (MG), the synaptic concentration of AChRs is disrupted by the circulation of anti-AChR antibodies generated in their own immune systems, leading to fluctuating muscle weakness and fatigability. However, the mechanisms underlying the endocytosis and degradation of AChRs from the postsynaptic sites in the pathogenesis of MG remain largely unknown. In this study, we aim to understand the molecular and cellular aspects on the cytoskeletal mechanisms underlying AChR endocytosis at the onset of MG pathogenesis. The results of this study should provide not only a better understanding of the fundamental mechanisms of NMJ development and maintenance, but also some valuable insights into the etiology of MG.

SPAIN

Barcelona - Universitat Pompeu Fabra Pura Munoz-Canoves Ph.D.

RG	CELLULAR MECHANISMS OF FIBROSIS DEVELOPMENT IN MUSCULAR DYSTROPHIES				
	\$130,000.00	2/1/2012	1/31/2013	Year 1	
	\$130,000.00	2/1/2013	1/31/2014	Year 2	
	\$130,000.00	2/1/2014	1/31/2015	Year 3	

Summary Our preliminary results show an exacerbated muscular dystrophy in mdx mice -model for DMD- lacking the protease inhibitor PAI-1, correlating with increased inflammation, fibrin deposition and fibrosis, whereas fibrin depletion attenuates disease progression. We propose that PAI-1 and fibrin may regulate inflammation-driven muscle degeneration and fibrosis development in muscular dystrophy through yet unknown mechanisms, which we aim to decipher in this project. Selective interference with fibrinogen anti-inflammatory functions, without affecting its blood clotting properties, is proposed as a potential new therapy for DMD, through combating fibrosis progression.

SWEDEN

Lund - Experimental Medical Science, Lund University Madeleine Durbeej-Hjalt Ph.D.

RG	Proteasome and autophagy inhibition in congenital muscular dystrophy			
	\$147,341.00	2/1/2012	1/31/2013	Year 2
	\$147.341.00	2/1/2013	1/31/2014	Year 3

Summary Congenital muscular dystrophy with laminin alpha2 chain deficiency (also known as MDC1A), is characterized by a decrease in muscle mass that partly may be caused by increased protein degradation. We hypothesize that blockade of protein degradation could be beneficial for MDC1A patients. Hence, we will perform pre-clinical studies in a mouse model for MDC1A. Mice will be treated with proteasome and autophagy inhibitors, respectively. These are drugs that block protein degradation in cells. Interestingly, we already have promising results using a proteasome inhibitor in laminin alpha2 chain deficient animals. We hope that our studies will generate important pre-clinical data for the development of pharmacological therapies for MDC1A patients.

UNITED KINGDOM

GLASGOW - University of Glasgow

Darren G. Monckton Ph.D.

RG Next generation sequencing approaches to genotyping in myotonic dystrophy type

1

\$145,000.00	2/1/2013	1/31/2014	Year 1
\$128,892.00	2/1/2014	1/31/2015	Year 2

Summary Myotonic dystrophy type 1 (DM1) varies from mild late onset to a very severe form that frequently results in the death of newborns. However, the genetic test for DM1 provides only a simple 'yes' or 'no' diagnosis and no information on likely disease severity, failing families in their ability to make informed life and reproductive choices. The inherited genetic material, DNA, contains the chemical 'letters' C, A, T and G arranged to form the 'instructions' or genes to build tissues. In the gene affected in DM1, the DNA letters CTG occur several times in a row, and in DM1 patients, the number is increased from 50 to 1000 or more in severely affected children. In patients the number of CTG repeats increases throughout life, making the symptoms worse. In the standard diagnostic test, this genetic instability is ignored and only an average number of repeats is measured. By analyzing the variation present in single cells we can provide more accurate predictions of disease severity. We have also shown that sometimes the CTGs are interrupted by other groups of three 'letters', such as CCG, that seem to be associated with milder symptoms. In this project we will collaborate with genetic testing laboratories to develop new diagnostic tests that will allow us both to detect variant repeats and to measure the variation in the number of CTGs present in patient DNA, improving advice available to families and facilitating more efficient clinical trials.

Francesco Muntoni M.D.

RG Uncovering the role of mitochondria in the pathogenesis of core myopathies \$125,000.00 7/1/2012 6/30/2013 Year

Summary Core myopathies, the most frequent congenital myopathy variants, are inherited muscle disorders characterized by weakness affecting the limb and trunk muscles. Examination of muscle biopsy reveals "core" areas completely devoid of mitochondria, surrounded by unaffected areas, inside the muscle cells. Mitochondria are structures that provide cells with the energy required for function. The genetic defect in core myopathies is represented by mutations in the muscle ryanodine receptor, the channel that mediates the calcium release required for contraction. While the absence of mitochondria from the muscle fibre "cores" is a key finding, it is not known how this relates to calcium dysregulation. However, there are strong, well established links between cellular calcium signals and mitochondrial bioenergetic function, biogenesis, free radical generation and movement. We therefore propose to study directly, in cell cultures developed from patient and control biopsies, how impaired calcium signals lead to the depletion of mitochondria within myofibers, with the overall goal to understand disease pathogenesis. Specifically, we propose to explore the relationships between cellular calcium signaling and mitochondrial biogenesis, bioenergetic function, autophagy, free radical generation and movement. We hope and expect that our results will clarify whether mitochondria can become therapeutic targets in these diseases, and possible in other related neuromuscular disorders.

London - Royal Veterinary College Susan Carol Brown PhD

RG An animal model for studying therapeutic approaches in FKRP related disease

Summary Mutations in any one of 6 genes leads to forms of muscular dystrophy collectively known as the 'dystroglycanopathies' the disease process of which is associated with a problem in the way alpha-dystroglycan is glycosylated or decorated with sugars. We previously generated mice which display a marked reduction in expression levels of Fukutin Related Protein or FKRP which is one of the genes that leads to a reduction in alpha dystroglycan glycosylation. These animals display a muscle, eye brain phenotype similar to that of patients with FKRP mutations at the severe end of the clinical spectrum. However, these animals die around the time of birth due to the reduction of FKRP in the central nervous system. In order to circumvent this we have now crossed these mice with lines that will replace FKRP in the CNS but not the muscle thus providing us with a model for LGMD2I. This model has an overt muscle pathology by 12 weeks of age and so will now be used to determine if some of the therapeutic approaches proposed for other forms of muscular dystrophy are appropriate for the dystroglycanopathies.

Northampton - International Alliance of ALS/MND Associations Rachel Patterson M.F.A.

SG The Allied Professionals Forum

\$5,000.00 12/5/2013 12/5/2013 Year 1

Summary The Allied Professionals Forum (APF) facilitates a discussion between health and social care professionals from all over the world who work with people living with ALS. The purpose of the APF is to encourage these professionals to share good practice, explaining the methods they have tried and the results and outcomes they have achieved. The APF fills an important gap in the professional conversation about ALS; rather than focusing only on research and medical studies, we are interested in practice-based evidence and in opening a discussion about the very real logistical issues that arise daily in caring for people with ALS. Each year, the APF invites health and social care providers to submit abstracts according to relevant themes. We select presentations that: demonstrate practical management strategies; provide opportunities to reflect on options and solutions; stimulate understanding and learning, as well as the ability to translate this into practice; and/or reflect on

international differences and similarities in care management. This year's themes include integration of palliative care; respiratory support and nutrition; responding to fronto-temporal dementia/lobar degeneration; caring for carers (facing issues dealing compassion fatigue and exhaustion); e-solutions to improve quality of life for people living with ALS; and more.

Oxford - University of Oxford

Kay Elizabeth Davies MA, Ph.D.,

RG	Screening for drugs to increase utrophin levels in DMD			
	\$130,000.00	2/1/2012	1/31/2013	Year 1
	\$130,000,00	2/1/2013	1/31/2014	Vear 2

\$130,000.00 2/1/2013 1/31/2014 Year 2 \$.00 2/1/2014 1/31/2015 Year 3

Summary Duchenne muscular dystrophy (DMD) is caused by the absence of the large protein called dystrophin in all muscle cells of patients. At present there is no effective treatment for the disorder, although there are several approaches in clinical trial. Our objective is to develop an effective small molecule drug therapy for DMD through increasing the amount of the dystrophin-related protein utrophin in muscle. This approach has the advantage of being applicable to all patients as it is not mutation-dependent. Furthermore, a small molecule drug orally administered can target all affected muscle types including heart and diaphragm.

Kay Elizabeth Davies MA, Ph.D.,

RG	Utrophin upregulation for treatment of DMD			
	\$103,783.00	2/1/2013	1/31/2014	Year 1
	\$103,783.00	2/1/2014	1/31/2015	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.

Staffordshire - Keele University

Glenn Eric Morris D. Phil.

RIG The MDA Monoclonal Antibody Resource for Neuromuscular Disease \$124,696.00 10/1/2012 9/30/2013 Year 3

Summary Specific antibodies are vital research tools in the fight against neuromuscular disease, but no single antibody can perform all the necessary functions. We have spent nearly 20 years in developing panels of large numbers of well-characterized antibodies for studies of the most common neuromuscular diseases (namely Duchenne/Becker and Emery-Dreifuss muscular dystrophies, spinal muscular atrophy and myotonic dystrophy). We have over 150 exon-specific dystrophin antibodies that are widely used in clinical trials of treatments for Duchenne dystrophy and in studies of animal models, as well as antibodies to distinguish different utrophin isoforms in utrophin upregulation studies. Our SMN antibodies have a significant role in the search for drugs that upregulate SMN in SMA patients. MDA funding enables us to maintain and develop this antibody resource. This involves characterization, promotion and distribution of existing panels of antibodies, and development of relevant new antibodies.