

2012 ACTIVE GRANTS

ALABAMA

Birmingham - The University of Alabama

Michael Miller, Ph.D.

(RG) A unifying molecular mechanism for familial amyotrophic lateral sclerosis

\$ 116,711 02/01/2011 - 01/31/2012 (Year 1)

\$ 116,711 02/01/2012 - 01/31/2013 (Year 2)

\$ 116,711 02/01/2013 - 01/31/2014 (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.

2012 ACTIVE GRANTS

ARIZONA

Phoenix - St. Joseph's Hospital & Medical Center

Fu-Dong Shi, M.D., Ph.D.

(RG) Th17 Cells in Myasthenia

\$	150,000	01/01/2010 - 12/31/2010	(Year 1)
\$	150,000	01/01/2011 - 12/31/2011	(Year 2)
\$	150,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: B cells are a fundamental component of the immune system. Anti-acetylcholine receptor (AChR)-antibodies are a product of B cells that cause myasthenia gravis (MG). However, these antibodies can form only with assistance from T helper (Th) cells and the cytokines they secrete, interferon and interleukin. Treatment with Rituximab (an antibody drug, which removes B cells from patients suffering from multiple sclerosis or other autoimmune disorders) has pioneered this research; however, this may not be an ideal approach for MG. A better approach for MG would be to interrupt only anti-AChR-producing B cells, while leaving other B cells and their functions intact. The best option for achieving this is to block the specific interactions between Th cells and B cells. When laboratory mice deficient in either Th1 or Th2 cytokines were manipulated to develop MG, the results were contradictory. A newly discovered subtype of cells, called Th17 cells, may be involved. However little is known about the effect of these Th17 cells on B cells and the diseases they cause. We aim to clarify the potential effects of Th17 cells and the cytokine IL-17 on B cell responses in a mouse model of human MG, experimental autoimmune MG (EAMG).

Phoenix - The Translational Genomics Research Institute

Lisa Baumbach, Ph.D.

(RG) Identification of New Disease Genes for Infantile Lower Motor Neuron Diseases

\$	129,076	08/01/2011 - 07/31/2012	(Year 1)
\$	129,076	08/01/2012 - 07/31/2013	(Year 2)
\$	129,076	08/01/2013 - 07/31/2014	(Year 3)

Summary: Our research group has led a long-term effort to identify and collect families with X-linked 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.

Tempe - Arizona State University

N. Jeanne Wilson-Rawls, Ph.D.

(RG) The Role of the Notch/Numb interaction in promoting skeletal muscle repair.

\$ 138,038 01/01/2009 – 12/31/2009 (Year 1)

\$ 117,152 01/01/2010 - 12/31/2010 (Year 2)

\$ 122,033 01/01/2011 – 06/30/2012 (Year 3)

Summary: Before any stem cell therapy can be incorporated into clinical practice it will be necessary to characterize the genetic and biochemical regulators of signals which drive stem cells to survive and self-renew. An understanding of the regulation of these events will represent an important step for enhancing the self-renewal of engrafted cells, and thus their regenerative potential as therapies for myopathies. Our studies ask important questions to determine the role of Numb regulation of Notch signaling and whether it creates a molecular switch between these two processes in myogenesis. Using inducible mutants of Numb we will examine the impact of this gene on satellite cell proliferation and differentiation. Muscle repair will be measured following experimentally induced injury by examining the rate and extent of muscle fiber regeneration and the number and distribution of active and reserve satellite cells. The establishment of genetically corrected populations of satellite cells represents a viable approach to treating some forms of muscular dystrophy. Enhancing the self-renewal capabilities of engrafted satellite cells will significantly contribute to the regenerative potential of this therapeutic approach.

Tucson - University of Arizona

Daniela Zarnescu, Ph.D.

(RG) Gene and Drug Discovery in a Drosophila Model of ALS

\$ 125,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 125,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 125,000 07/01/2012 - 06/30/2013 (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.

2012 ACTIVE GRANTS

CALIFORNIA

Berkeley - University of California

Jen-Chywan Wang, Ph.D

(RG) Mechanisms of Glucocorticoid-induced Muscle Atrophy
\$ 110,000 02/01/2011 - 01/31/2012 (Year 1)
\$ 110,000 02/01/2012 - 01/31/2013 (Year 2)

Summary: Duchenne Muscular Dystrophy (DMD) is an incurable genetic disease affecting one in 3500 males in the . 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 - University of California

Gino Cortopassi, Ph.D.

(RG) Screening for compounds that improve mitochondrial disease outcome
\$ 86,800 01/01/2010 - 12/31/2010 (Year 1)
\$ 88,969 01/01/2011 - 12/31/2011 (Year 2)
\$ 91,224 01/01/2012 - 12/31/2012 (Year 3)

Summary: Inherited mitochondrial disease results in neurodegeneration (LHON,FRDA) and movement and muscle disorders (MERRF, MELAS,CPEO) in thousands of Americans. We have recently improved an assay with which to screen thousands of compounds for their positive effect on mitochondrial function, based on mitochondrial oxygen consumption. We have demonstrated that known enhancers of mitochondrial function operating through the PGC1-alpha pathway are detected by the assay. We have accumulated several cell models of mitochondrial disease, i.e. cells bearing the specific point mutations or deletions that cause the most prevalent mitochondrial diseases (LHON, CPEO, or nuclear defects in frataxin that cause Friedreich's ataxia). We demonstrate, using the assay, that cells bearing mutations causing mitochondrial disease have defects in mitochondrial oxygen consumption. Thus we will screen large drug libraries to identify compounds that enhance the function of mitochondria

compromised by mutations that cause mitochondrial disease. These compounds may serve as leads for mitochondrial disease therapy.

Michael Ferns, Ph.D.

(RG) Regulation of AChR Turnover and Implications for Diseases of NMJ

\$ 125,565 07/01/2009 – 06/30/2010 (Year 1)

\$ 125,565 07/01/2010 - 06/30/2011 (Year 2)

\$ 125,565 07/01/2011 - 06/30/2012 (Year 3)

Summary: At the neuromuscular junction (NMJ), the acetylcholine receptor (AChR) is aggregated at high density in the postsynaptic membrane, ensuring that synaptic transmission is rapid and reliable. The AChR is localized by interactions with postsynaptic scaffolding proteins, regulated in large part by a motoneuron-derived factor called agrin. Receptor levels are also maintained by metabolic stabilization of the AChR due to unknown mechanisms that reduce its turnover at the synapse. In myasthenia gravis, however, patients commonly develop autoantibodies to the AChR, which reduce AChR levels and impair transmission, resulting in severe muscle weakness. We are interested in defining the mechanisms that regulate AChR turnover and understanding how these processes are affected in myasthenia gravis. We hypothesize that AChR turnover is mediated in part by intrinsic signals for receptor endocytosis and degradation. Our specific aims are: (1) to define the endocytic motifs that mediate internalization of AChR and to determine how they are regulated by neural signals like agrin; and (2) to test whether antibody-induced AChR turnover in myasthenia gravis is mediated by the same endocytic signals and if it can be inhibited by mutations of the motifs or by agrin. These studies will define the molecular mechanism of normal and antibody-induced AChR turnover and may suggest new therapeutic strategies to maintain functional levels of receptor in myasthenia gravis and other diseases of the NMJ.

Samantha Harris, Ph.D.

(RG) Regulation of skeletal muscle contraction by myosin binding protein-C

\$ 122,012 08/01/2011 - 07/31/2012 (Year 1)

\$ 122,012 08/01/2012 - 07/31/2013 (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.

La Jolla - Ludwig Institute for Cancer Research

Don Cleveland, Ph.D.

(RG) Determining the contribution of mitochondrial dysfunction in ALS pathogenesis

\$	142,303	08/01/2011 - 07/31/2012	(Year 1)
\$	153,699	08/01/2012 - 07/31/2013	(Year 2)
\$	133,981	08/01/2013 - 07/31/2014	(Year 3)

Summary: Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative 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	02/01/2011 - 01/31/2012	(Year 1)
\$	60,000	02/01/2012 - 01/31/2013	(Year 2)
\$	60,000	02/01/2013 - 01/31/2014	(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	08/01/2011 - 07/31/2012	(Year 1)
\$	60,000	08/01/2012 - 07/31/2013	(Year 2)
\$	60,000	08/01/2013 - 07/31/2014	(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

Anne Bang, Ph.D.

(RRG) Restricted Funds for Research - McCaw Fund

\$	50,000	11/01/2011 – 10/31/2012	(Year 1)
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Pier Lorenzo Puri, M.D. Ph. D.

(RG) Signal-dependent control of gene expression in satellite cells

\$	103,112	02/01/2011 - 01/31/2012	(Year 1)
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\$	103,112	02/01/2012 - 01/31/2013	(Year 2)
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\$	103,112	02/01/2013 - 01/31/2014	(Year 3)
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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

\$	149,226	08/01/2011 - 07/31/2012	(Year 1)
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\$	148,777	08/01/2012 - 07/31/2013	(Year 2)
\$	148,777	08/01/2013 - 07/31/2014	(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 Scripps Research Institute

Priti Phukan Deka, Ph. D.

(DG) Structural and Mechanistic Role of Muscleblind Protein in Myotonic Dystrophy

\$	60,000	01/01/2010 - 12/31/2010	(Year 1)
\$	60,000	01/01/2011 - 12/31/2011	(Year 2)
\$	60,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: Muscleblind (MBNL) is an RNA-binding protein that has been closely linked to myotonic dystrophy (DM). MBNL binds single-stranded RNA under non-pathological conditions and plays a key role in normal muscle development. In myotonic dystrophy, the MBNL protein is sequestered by pathogenic RNA containing expanded CUG and CCUG repeats. This sequestration of the MBNL protein results in inhibition of its normal function as a regulator of alternative RNA splicing, leading to DM. We aim to study the mechanism by which the muscleblind protein recognizes specific regulatory sites in single-stranded RNA under normal conditions, and also binds with high affinity to pathogenic CUG and CCUG repeat hairpin RNAs. Upon understanding the molecular basis for these interactions, we will design a strategy to inhibit the interaction of MBNL with pathogenic RNA hairpins, in order to develop a potential DM therapy. We will do this by screening a library of small molecules for compounds that inhibit binding of muscleblind protein to pathogenic RNAs.

La Jolla - University of California, San Diego

Ju Chen, Ph.D.

(RG) The Role of Cypher in skeletal muscle function and disease

\$	110,000	07/01/2010 - 06/30/2011	(Year 1)
\$	110,000	07/01/2011 - 06/30/2012	(Year 2)
\$	110,000	07/01/2012 - 06/30/2013	(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.

Masahiko Hoshijima, M.D., Ph.D.

(RG) Genetic treatment of cardio-respiratory failure in muscular dystrophy

\$ 122,462 02/01/2011 - 01/31/2012 (Year 1)

\$ 122,462 02/01/2012 - 01/31/2013 (Year 2)

\$ 122,462 02/01/2013 - 01/31/2014 (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 07/01/2010 - 06/30/2011 (Year 1)

\$ 110,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 110,000 07/01/2012 - 06/30/2013 (Year 3)

Summary: 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.

Alysson Muotri, Ph.D.

(RG) Modeling Familial ALS8 with Human Induced Pluripotent Stem Cells

\$ 120,822 02/01/2011 - 01/31/2012 (Year 1)

\$ 120,822 02/01/2012 - 01/31/2013 (Year 2)

\$ 120,822 02/01/2013 - 01/31/2014 (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.

Los Angeles - Cedars-Sinai Medical Center

Robert Baloh, M.D., Ph.D.

(RG) Characterization of a TDP43 mouse model of motor neuron disease

\$ 142,407 07/01/2009 - 06/30/2010 (Year 1)

\$ 133,278 07/01/2010 - 06/30/2011 (Year 2)

\$ 135,172 07/01/2011 - 06/30/2012 (Year 3)

Summary: In ~10% of ALS patients the disease runs in their family. Studying these families has led to the discovery of genes that can cause ALS, and can be used to develop animal models of the disease. This pathway of research led to the development of the SOD1 mouse model, which is a mainstay for testing new therapies for ALS. Our group and others recently found that mutations in a gene called TDP-43 are another important cause of familial ALS. This is exciting because fragments of the TDP-43 protein are found in the motor neurons in most forms of ALS, including the more common form that does not run in families. Therefore, it is likely that understanding how TDP-43 gene mutations cause ALS will provide completely new insights into how the disease develops. We have continued our work by engineering a mouse which expresses the mutated form of TDP-43 that we discovered in a family with ALS followed at the MDA clinic at Washington University. Our initial evaluation of this mouse shows that it develops a progressive and fatal disease with similarity to ALS. This grant

proposal outlines experiments to characterize this mouse model in detail, so that it can effectively be used to develop and test new therapies for ALS.

Ronald Victor, MD

(RG) Functional muscle ischemia and PDE5A inhibition in Becker muscular dystrophy

\$ 311,579 01/01/2010 - 12/31/2010 (Year 1)

\$ 311,579 01/01/2011 - 12/31/2011 (Year 2)

\$ 311,579 01/01/2012 - 12/31/2012 (Year 3)

Summary: We will conduct new patient-centered research that aims to find effective treatment for Duchenne muscular dystrophy. Our study is based on a recent research finding that Viagra and other drugs for erectile dysfunction benefit muscular dystrophy by improving blood flow to the diseased muscles and reducing fatigue. However, that research was performed in mice; our studies will be done in patients with muscular dystrophy. We will start with adult patients, who are less ill than children with muscular dystrophy and will have an easier time participating in the research, which will include exercise testing, measurements of muscle blood flow and oxygen delivery, and magnetic resonance imaging of the muscles. If the results are positive, the studies could be modified to include children. The hope is that this patient-oriented research will suggest a new use of existing drugs that could quickly improve the treatment of BMD and DMD.

Los Angeles - University of California

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

(RG) Novel Mechanisms to Enhance Utrophin Expression and Muscle Cell Function

\$ 120,228 07/01/2009 - 06/30/2010 (Year 1)

\$ 122,259 07/01/2010 - 06/30/2011 (Year 2)

\$ 116,170 07/01/2011 - 06/30/2012 (Year 3)

Summary: Loss of functional dystrophin protein in Duchenne muscular dystrophy results in reduced muscle membrane stability, with myocyte death and muscle 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 taken a broad approach to identify molecules that stabilize the sarcolemma by enhancing cell attachment. As a result, we have discovered a novel compound that improves sarcolemma stability and adhesion. Importantly, this is a FDA-approved compound that has the potential to treat DMD and other congenital muscular dystrophies. The current proposal is aimed at testing the mechanisms and feasibility of this novel approach in murine models of muscular dystrophy.

Carmen Bertoni, Ph.D.

(MVP) Preclinical Investigation of RTC#13 for the Treatment of DMD

\$ 246,483 08/01/2010 - 07/31/2011 (Year 1)

\$ 153,725 08/01/2011 - 07/31/2012 (Year 2)

\$ 76,257 08/01/2012 - 07/31/2013 (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) Dystrophin Gene Editing Strategies for Duchenne Muscular Dystrophy

\$	136,305	07/01/2010 - 06/30/2011	(Year 1)
\$	136,305	07/01/2011 - 06/30/2012	(Year 2)
\$	136,305	07/01/2012 - 06/30/2013	(Year 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.

Giovanni Coppola, MD

(RG) Peripheral Biomarkers in Friedreich's Ataxia

\$	132,847	02/01/2011 - 01/31/2012	(Year 1)
\$	132,847	02/01/2012 - 01/31/2013	(Year 2)
\$	132,847	02/01/2013 - 01/31/2014	(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.

Melissa Spencer, Ph.D.

(RG) Investigation of osteopontin and inflammatory processes in mdx mice

\$	125,000	08/01/2011 - 07/31/2012	(Year 1)
\$	125,000	08/01/2012 - 07/31/2013	(Year 2)
\$	125,000	08/01/2013 - 07/31/2014	(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 targeting 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.

James Tidball, Ph.D.

(RG) Regenerative mechanisms driven by M2 macrophages in dystrophic muscle.

\$	150,344	01/01/2010 - 12/31/2010	(Year 1)
\$	149,435	01/01/2011 - 12/31/2011	(Year 2)
\$	130,705	01/01/2012 - 12/31/2012	(Year 3)

Summary: Muscle inflammation is a prominent feature of Duchenne muscular dystrophy. Inflammatory cells can play an important role in influencing the progress of pathology in dystrophic muscle. Our investigation is directed toward identifying the specific mechanisms through which inflammatory cells promote muscle regeneration in muscular dystrophy, so that those beneficial functions can be amplified therapeutically.

Los Angeles - USC/University of Southern California

Pragna I. Patel, Ph.D.

(RG) Molecular genetics of autoimmune myasthenia gravis

\$	121,139	02/01/2011 - 01/31/2012	(Year 1)
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Summary: Myasthenia gravis is an autoimmune disease with weakness of the skeletal muscles. Muscles that control eye and eyelid movement, facial expression, chewing, talking, swallowing, breathing and neck and limb movements, can be affected. We have studied a rare family where 10 individuals are affected, and will identify the gene defective in it. In the future, we will examine the role of this new gene, and genes that work together with it in sporadic MG cases. These studies will lay the foundation for novel treatment strategies since none of the treatments used currently are optimal, can have side effects and provide temporary relief.

Merced - University of California

Wei-Chun Chin, Ph.D.

(RG) Carbon Nanotubes Based Polymer Scaffolding for Neuron Regeneration

\$	109,784	01/01/2010 - 12/31/2010	(Year 1)
\$	109,784	01/01/2011 - 12/31/2011	(Year 2)
\$	109,784	01/01/2012 - 12/31/2012	(Year 3)

Summary: The fact that our bodies are not capable of regenerating axons and re-innervating target cells makes embryonic stem cell-based transplant therapy a feasible and promising approach for muscular dystrophy patients. Carbon nanotubes (CNTs) with length and diameter on the scale of extracellular matrix (ECM) components such as collagen or fibronectin that can interact with stem cells and have significant influence on stem cell differentiation/fate. CNTs have been considered as an ideal candidate for scaffolds in tissue engineering applications. Other than their unique ability to conduct electricity and direct neuron growth, CNTs are also elastic with mechanical integrity, and may offer roughness on substrates for ECM absorption. These unique advantages make CNTs an ideal material for neuronal-lineage-oriented human Embryonic Stem Cells (hESCs) scaffolds. Our preliminary data have indicated PMAA (polymethacrylic acid) coated CNTs can efficiently induce neuronal differentiation. In this proposed project, we plan to develop 3D CNT-based polymer scaffolds to promote better neuron differentiation efficiency from hESCs for motor neuron repairs or neuron-transplants for neuromuscular disease.

Oakland - Children's Hospital & Research Center Oakland

Julie Saba, M.D.

(RG) Sphingosine-1-phosphate signaling in muscle regeneration and homeostasis

\$	132,005	02/01/2012 - 01/31/2013	(Year 1)
\$	130,231	02/01/2013 - 01/31/2014	(Year 2)
\$	130,231	02/01/2014 - 01/31/2015	(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.

Orange - University of California (Irvine)

Virginia Kimonis, M.D., MRCP

(RG) Preclinical Studies in the VCP knock-in mouse model of hIBM

\$	124,000	07/01/2010 - 06/30/2011	(Year 1)
\$	124,000	07/01/2011 - 06/30/2012	(Year 2)

\$ 124,000 07/01/2012 - 06/30/2013 (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.

Palo Alto - Palo Alto Institute for Research & Education, Inc.

Thomas Rando, M.D., Ph.D.

(RG) Mechanisms of Fibrosis in Muscular Dystrophies

\$ 125,000 08/01/2011 - 07/31/2012 (Year 1)

\$ 125,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 125,000 08/01/2013 - 07/31/2014 (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.

Redwood City - Glialogix, Inc.

Mark Moore, Ph.D.

(MVP) Investigation of GLX1112, a Potential Pharmacological Agent for the Treatment of Amyotrophic Lateral Sclerosis, Using the SOD1G93A Mouse, a Standard Transgenic Mouse Model of ALS

\$ 50,250 11/1/2010 - 12/31/2010 (Year 1)

\$ 217,750 1/1/2011 - 1/31/2012 (Year 2)

Summary: Glialogix has identified a novel drug target for antiglutamatergic therapy in neurodegenerative diseases, including ALS. Studies in ALS patients and mouse models of ALS have shown elevated glutamatergic signaling, and inhibition of excessive

glutamatergic signaling has been clinically validated for both Alzheimers and ALS. Our target releases glutamate into the extracellular space, thereby increasing signaling through all glutamate receptors. Glialogix's target is activated in models of Parkinson's and Alzheimers especially in the microglia, and has been shown to be responsible for increasing the level of glutamate in the brain. We propose to confirm that this target is also activated in ALS patients and mouse models, and will antagonize that activation with a clinical compound (GLX1112) that we have also identified. We plan to further validate the CNS access, therapeutic mechanism and safety of GLX1 112.

San Diego - San Diego State University Research Foundation

Sanford Bernstein, Ph.D.

(RG) Disease mechanism and therapy development for inclusion body myopathy type 3

\$ 123,437 02/01/2012 - 01/31/2013 (Year 1)

\$ 123,437 02/01/2013 - 01/31/2014 (Year 2)

\$ 123,437 02/01/2014 - 01/31/2015 (Year 3)

Summary: Dominant hereditary inclusion body myopathy type 3 (IBM-3) is caused by a mutation in 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 - University of California

Harold Bernstein, M.D., Ph.D.

(RG) Analysis of human muscle stem cells: Toward therapy for muscular dystrophies

\$ 180,000 02/01/2011 - 01/31/2012 (Year 1)

\$ 180,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 180,000 02/01/2013 - 01/31/2014 (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.

Douglas Gould, Ph.D.

(RG) Interrogation of a novel genetic model of muscle-eye-brain disease.

\$	144,056	01/01/2010 - 12/31/2010	(Year 1)
\$	144,056	01/01/2011 - 12/31/2011	(Year 2)
\$	142,802	01/01/2012 - 12/31/2012	(Year 3)

Summary. Genetic approaches have been successful in determining the causes of congenital muscular dystrophies. Identifying the underlying genes, and using mouse models to understand how mutant genes cause disease, can lead to development of new treatments and therapies. Mice with a mutation in the type IV collagen alpha 1 gene (Col4a1) have abnormalities observed in patients with Muscle-Eye-Brain disease and other associated congenital muscular dystrophies. We will test to see if COL4A1 is involved in human disease and seek to understand the cellular mechanisms that lead from mutation to disease.

Eric Huang, M.D., Ph.D.

(RG) Mechanisms of FUS mutations in motor neuron survival and synaptogenesis

\$	137,500	02/01/2012 - 01/31/2013	(Year 1)
\$	137,500	02/01/2013 - 01/31/2014	(Year 2)
\$	137,500	02/01/2014 - 01/31/2015	(Year 3)

Summary. ALS is a fatal disease 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.

Jianming Liu, Ph.D

(DG) Targeting Smad mediated signaling of TGFbeta family for stem cell therapy of DMD

\$	59,986	02/01/2011 - 01/31/2012	(Year 1)
\$	59,929	02/01/2012 - 01/31/2013	(Year 2)
\$	59,997	02/01/2013 - 01/31/2014	(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 effector 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.

Santa Cruz - University of California

Manuel Ares, Ph.D.

(RG) Developing a diagnostic tool for splicing misregulation in DM1 and DM2

\$ 100,000 07/01/2009 – 06/30/2010 (Year 1)

\$ 100,000 07/01/2010 - 06/30/2011 (Year 2)

\$ 100,000 07/01/2011 - 06/30/2012 (Year 3)

Summary: The mutations that lead to myotonic dystrophy (DM1 and DM2) cause accumulation of toxic RNA in the cell nucleus, binding up splicing factors including MBNL1, and causing gene expression perturbations. Splicing is an essential step in gene expression. With expression of the toxic RNA, pre-mRNAs that normally depend on MBNL1 become abnormally spliced, leading to faulty proteins and disease. We found disease-related mis-splicing events by using splicing-sensitive microarrays and two mouse models of DM1: one over-expresses CUG-RNA; the other does not make MBNL1. We find practically the same the splicing changes in these two genetically distinct mice, implying that CUG-RNA expression leads to loss of MBNL1 function. We observed many of these same splicing changes in human DM. We have also identified a class of genes whose expression is altered in the CUG-RNA expressing mouse but that are not dependent on MBNL1. We propose to develop a diagnostic tool to measure gene expression events affected in DM1 and DM2. We will make oligonucleotide sets for "RASL", a ligation-based nucleic acid detection method that specifically measures splicing using small amounts of RNA. This will enable the measurement of hundreds of DM-related splicing and gene expression events simultaneously, providing a rich set of biomarker data to illuminate issues of disease progression, disease severity, patient genetic background, and outcomes of therapy, while minimizing pain to patients.

Stanford - Stanford University

Michele Calos, Ph.D.

(RG) Stem cell therapy for Duchenne muscular dystrophy

\$ 131,299 07/01/2010 - 06/30/2011 (Year 1)

\$ 100,000 07/01/2011 - 06/30/2012 (Year 2)

Summary: Our goal is to use stem cells obtained from patients to create functional muscle fibers in muscles damaged by Duchenne muscular dystrophy (DMD). In these proof of principle experiments, stem cells will be harvested from mice with DMD. A good copy of the gene mutated in DMD (dystrophin) will be added to the cells. Then the corrected cells will be returned to the mouse, where they will home to damaged muscle and regenerate intact muscle fibers. We will use an abundant type of adult stem cell that can be purified from fat tissue and has been shown to develop into muscle (adipose-derived mesenchymal stem cells). DNA carrying the therapeutic gene will be added to the cells using safe, non-viral methods. The DNA will become a permanent part of the chromosomes and will be integrated in a biologically secure location. The corrected stem cells will be injected back into the mouse. The percentage of healthy

fibers that form in various muscles will be measured. We will also work with the equivalent human stem cells, derived from fat tissue, in order to optimize conditions for work with human cells. The safety of the integration sites will be examined, and it will be verified that the cells do not cause cancer. Success in these experiments will lead to future studies in DMD dogs, preparatory to clinical trials.

Andrew Tri Van Ho, Ph.D.

(DG) Treatment of mdx/mTR Model of DMD with Human Muscle Stem Cells

\$	60,000	02/01/2012 - 01/31/2013	(Year 1)
\$	60,000	02/01/2013 - 01/31/2014	(Year 2)
\$	60,000	02/01/2014 - 01/31/2015	(Year 3)

Summary: Duchenne Muscular Dystrophy (DMD) is a fatal genetic disease characterized by progressive loss of skeletal muscle mass and function combined with heart muscle disease. Current therapeutic approaches 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 proposal, we plan to develop isolation procedures and to 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 Nelson, Ph.D.

(RG) Gene-Environment Interactions in the Etiology of ALS

\$	98,209	01/01/2009 - 12/31/2009	(Year 1)
\$	92,107	01/01/2010 - 12/31/2010	(Year 2)
\$	93,494	01/01/2011 - 03/31/2012	(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	02/01/2012 - 01/31/2013	(Year 1)
\$	102,687	02/01/2013 - 01/31/2014	(Year 2)
\$	102,687	02/01/2014 - 01/31/2015	(Year 3)

Summary: In collaboration with Jeff Chamberlain and Helen Blau, 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, (Mol Ther. 2010 Mar;18(3):617-24). In studies by Mendell and colleagues, "delivery of a functional dystrophin transgene to skeletal muscle in six patients with Duchenne's muscular dystrophy. Dystrophin-specific T cells were detected after treatment", N Engl J Med. 2010 Oct 7;363(15):1429-37. We have developed a technology with an engineered DNA vaccine to induce tolerance to self-proteins and to foreign proteins [Nat Biotechnol. 2003 Sep;21(9):1033-9; J Intern Med. 2010 May;267(5):441-51]. 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. The PI's have collaborated on clinical trials in DMD with myoblasts [Nature. 1992 Apr 2;356(6368):435-8], and have studied the immune response in Duchenne's muscle.

2012 ACTIVE GRANTS

COLORADO

Aurora - University of Colorado Denver, AMC and DC

Kurt Beam, Ph.D.

(RG)	Voltage sensor for excitation-contraction coupling		
\$	101,146	07/01/2010 - 06/30/2011	(Year 1)
\$	101,146	07/01/2011 - 06/30/2012	(Year 2)
\$	101,146	07/01/2012 - 06/30/2013	(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.

William Betz, Ph.D.

(RG)	NMJ presynaptic function in a mouse transgenic with synaptotagmin		
\$	147,167	01/01/2010 - 12/31/2010	(Year 1)
\$	97,578	01/01/2011 - 12/31/2011	(Year 2)
\$	97,578	01/01/2012 - 12/31/2012	(Year 3)

Summary: Skeletal muscle fibers are stimulated to contract by acetylcholine (ACh), which is secreted by nerve terminals at the neuromuscular synapse. The ACh is contained in vesicles inside the terminals, like tiny soap bubbles (vesicles) inside a big soap bubble (the terminal). Previously, we have characterized a new genetically engineered mouse possessing terminals that 'light up' when stimulated. We will now use this synaptotagmin (spH) mouse to extend our previous studies to a molecular level, examining the relationship between sites of ACh secretion and locations of specialized 'active zones' from which most or all secretion is thought to occur. The neuromuscular synapse of the spH mouse is uniquely suited for these studies; they are not possible to perform in any other preparation.

Boulder - University of Colorado

Bradley Olwin, Ph.D.

(RG)	Identification and Characterization of Satellite Stem Cells		
\$	123,055	02/01/2011 - 01/31/2012	(Year 1)
\$	123,055	02/01/2012 - 01/31/2013	(Year 2)
\$	123,055	02/01/2013 - 01/31/2014	(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.

2012 ACTIVE GRANTS

CONNECTICUT

New Haven - Yale University

Hao Shi, Ph.D.

(DG)	Role of MKP-5 in Duchenne Muscular Dystrophy		
\$	60,000	02/01/2011 - 01/31/2012	(Year 1)
\$	60,000	02/01/2012 - 01/31/2013	(Year 2)
\$	60,000	02/01/2013 - 01/31/2014	(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.

Storrs - University of Connecticut

David Goldhamer, Ph.D.

(RG)	Regulation of satellite cell lineage commitment in regeneration and disease		
\$	125,000	02/01/2011 - 01/31/2012	(Year 1)
\$	125,000	02/01/2012 - 01/31/2013	(Year 2)
\$	125,000	02/01/2013 - 01/31/2014	(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.

2012 ACTIVE GRANTS

DISTRICT OF COLUMBIA

Washington - Children's Research Institute (CNMC)

Eric Hoffman, Ph.D.

(RG)	Mechanism of steroid action in DMD		
\$	98,557	01/01/2010 - 12/31/2010	(Year 1)
\$	98,557	01/01/2011 - 12/31/2011	(Year 2)
\$	98,557	01/01/2012 - 12/31/2012	(Year 3)

Summary: Glucocorticoids are considered standard of care in Duchenne muscular dystrophy. These drugs have complex pharmacological properties (potency, pharmacodynamics, pharmacokinetics). Importantly, prednisone has a deleterious effect on normal muscle function (steroid myopathy), yet is beneficial to Duchenne muscle. Our research is focused on understanding the effects of glucocorticoids on muscle, with the goal of enabling the development of better-targeted and more effective therapies for Duchenne muscular dystrophy.

Terence Partridge, Ph.D.

(RG)	Measuring the dynamics of muscle growth and disease in the mouse model of DMD		
\$	125,000	08/01/2011 - 07/31/2012	(Year 1)
\$	125,000	08/01/2012 - 07/31/2013	(Year 2)
\$	125,000	08/01/2013 - 07/31/2014	(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.

Christopher Spurney, M.D.

(RG)	Role of Toll-like receptors in the cardiomyopathy of dystrophin deficient mice		
\$	101,355	01/01/2010 - 12/31/2010	(Year 1)

\$	82,308	01/01/2011 - 12/31/2011	(Year 2)
\$	83,289	01/01/2012 - 12/31/2012	(Year 3)

Summary: The mechanisms underlying the progressive weakness and fibrosis in dystrophin deficient muscles are not known. The response of muscle cells to the loss of dystrophin includes membrane instability, calcium dysregulation and inflammation. Inflammation is mediated by toll-like receptors in many different cell types, including cardiac and skeletal muscle cells. The central hypothesis of these studies is that MyD88-dependent Toll-like receptor (TLR) signaling modulates the levels of reactive oxygen species and NF-kB activation, leading to muscle cell death and fibrosis. We have already shown improved skeletal and cardiac muscle function in a small number of MyD88 deficient mdx mice. The specific aims of this project expand on these findings and: 1) Study the role of MyD88 dependent TLR signaling in the progression of cardiac muscle disease in dystrophin deficient mice; 2) Study the role of MyD88 dependent TLR signaling in the progression of skeletal/ diaphragm muscle disease in dystrophin deficient mice; 3) Use modified morpholino oligomers to silence MyD88 signaling in mdx mice in an attempt to prevent the development of muscular weakness and fibrosis. The results from this work will be directly relevant toward understanding mechanisms involved in muscular pathology of dystrophin deficient mice and potentially identify novel therapies to prevent the progression of muscle disease in DMD.

2012 ACTIVE GRANTS

FLORIDA

Gainesville - University of Florida

Celine Baligand, Ph.D.

(DG) MRI/MRS evaluation of muscle function and treatment strategies in Pompe disease

\$ 59,817 02/01/2012 - 01/31/2013 (Year 1)

\$ 57,105 02/01/2013 - 01/31/2014 (Year 2)

\$ 53,951 02/01/2014 - 01/31/2015 (Year 3)

Summary: Pompe disease is a rapidly progressive muscular dystrophy caused by a deficiency in 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 02/01/2012 - 01/31/2013 (Year 1)

\$ 59,925 02/01/2013 - 01/31/2014 (Year 2)

\$ 59,925 02/01/2014 - 01/31/2015 (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. Without treatment, infants may die within 1-2 years of life. 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		
\$	59,617	07/01/2010 - 06/30/2011	(Year 1)
\$	59,594	07/01/2011 - 06/30/2012	(Year 2)
\$	59,499	07/01/2012 - 06/30/2013	(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 Ranum, Ph.D.,

(RG)	Molecular Effects of Repeat Associated Non-ATG Translation in Myotonic Dystrophy		
\$	138,364	02/01/2012 - 01/31/2013	(Year 1)
\$	138,364	02/01/2013 - 01/31/2014	(Year 2)
\$	138,364	02/01/2014 - 01/31/2015	(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.

Jupiter - The Scripps Research Institute

Matthew Disney, Ph.D.

(RG)	Targeting RNAs that Cause Myotonic Muscular Dystrophy with Small Molecules		
\$	65,087	01/01/2010 - 12/31/2010	(Year 1)
\$	68,580	01/01/2011 - 12/31/2011	(Year 2)
\$	71,492	01/01/2012 - 12/31/2012	(Year 3)

Summary: In myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2), toxic repeating RNAs fold into a hairpin-like structure that binds the protein muscleblind. Sequestration of muscleblind by the RNA hairpins causes defects in a host of proteins, including the main muscle chloride channel and the insulin receptor. To better harness this information and turn it into DM therapies, this project will study the potential of designed, cell permeable small molecules to target the toxic RNA-protein interaction that causes DM and correct defects in the main chloride muscle channel and the insulin receptor in DM cells. If successful, these compounds could serve as the first treatments for DM.

Miami - University of Miami

Ellen Barrett, Ph.D.

(RG) Preserving motor nerve terminals in mouse models of familial ALS

\$	99,034	02/01/2011 - 01/31/2012	(Year 1)
\$	99,034	02/01/2012 - 01/31/2013	(Year 2)
\$	99,034	02/01/2013 - 01/31/2014	(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 drug-treated 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.

Antoni Barrientos, Ph.D.

(RG) Role of New Evolutionary Conserved Cytochrome c Oxidase Assembly Chaperones

\$	115,500	02/01/2011 - 01/31/2012	(Year 1)
\$	115,500	02/01/2012 - 01/31/2013	(Year 2)
\$	115,500	02/01/2013 - 01/31/2014	(Year 3)

Summary: Cytochrome c oxidase (COX) deficiency is the most frequent cause of mitochondrial 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.

Michael Benatar, M.D., Ph.D., PhD

(RG) Pre-familial ALS (Pre-fALS) Study

\$	175,000	07/01/2010 - 06/30/2011	(Year 1)
\$	175,000	07/01/2011 - 06/30/2012	(Year 2)
\$	175,000	07/01/2012 - 06/30/2013	(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.

Flavia Fontanesi, Ph.D

(DG) Basis of Cytochrome c Oxidase defects in primary mitochondrial ATPase deficiency

\$	60,000	01/01/2010 - 12/31/2010	(Year 1)
\$	60,000	01/01/2011 - 12/31/2011	(Year 2)
\$	60,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: ATP synthase, or ATPase, is a key enzyme for energy production in mitochondria. Defects in the ATPase cause rare, very severe mitochondrial encephalomyopathies, frequently associated with decrease in cytochrome c oxidase (COX), the last enzyme of the mitochondrial respiratory chain. We will investigate the molecular mechanisms responsible for the defect in COX biogenesis observed in ATPase deficient cells. Since a similar COX biogenesis defect was observed in yeast ATPase mutants will use genetic and pharmacological yeast and human cell models of ATPase deficiencies.

Carlos Moraes, Ph.D.

(RG) Increased Mitochondrial Biogenesis as Therapy to Mitochondrial Disorders

\$	121,224	07/01/2010 - 06/30/2011	(Year 1)
\$	121,224	07/01/2011 - 06/30/2012	(Year 2)
\$	121,224	07/01/2012 - 06/30/2013	(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.

2012 ACTIVE GRANTS

GEORGIA

Atlanta - Emory University

Luciano Apponi, Ph.D.

(DG) Mechanisms of Muscle Specificity in OPMD

\$	60,000	01/01/2010 - 12/31/2010	(Year 1)
\$	60,000	01/01/2011 - 12/31/2011	(Year 2)
\$	60,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: Oculopharyngeal muscular dystrophy (OPMD) is an adult onset disease characterized by eyelid drooping, difficulties in swallowing and weakness in limb muscles. Although alanine expansion in PABPN1, a ubiquitous mRNA binding protein, causes OPMD, how mutant PABPN1 leads to pathology in skeletal muscle is unknown. Our main goal is to understand why only muscle is affected in OPMD by exploring hypotheses related to PABPN1 expression and function. Eukaryotic mRNA is characterized by the addition of several hundred adenosines to the end of the molecule, generating a poly(A) tail; poly(A) tail length is critical for gene expression. PABPN1 modulates poly(A) tail length, and our preliminary studies show PABPN1 loss in cultured muscle cells leads to mRNA with shorter poly(A) tails. Thus, the expression of mutant PABPN1 could change expression of genes important for muscle function by deregulating poly(A) tail length. In Specific Aim 1, we will test whether mutant PABPN1 alters poly(A) tail length in mouse muscle. Our preliminary results demonstrate that in skeletal muscle PABPN1 mRNA and protein levels are much lower than in other tissues, potentially making muscle more vulnerable to the effects of mutant PABPN1. In Specific Aim 2, we will define the elements involved in regulating PABPN1 expression in muscle.

Gary Bassell, Ph.D.

(RG) Axonal mRNA regulation by hnRNP-Q1 and SMN

\$	100,679	01/01/2009 - 12/31/2009	(Year 1)
\$	98,555	01/01/2010 - 12/31/2010	(Year 2)
\$	101,555	01/01/2011 - 03/31/2012	(Year 3)

Summary: An important objective of spinal muscular atrophy research is to understand the normal functions of the Survivor of Motor Neuron (SMN) protein in the nervous system. SMN is transported in RNA granules which appear to contain mRNAs that are locally translated within axons. Local protein synthesis within axons provides an important mechanism for the axon to control its own structure and function during development and possibly also within mature nerves. Thus, it has been hypothesized that SMN may play some role in the mechanism of mRNA transport and local protein synthesis. A major objective of this project is to identify SMN protein partners in axons. Preliminary data indicate that the SMN interacting protein, hnRNP-Q1, is co-transported with SMN in motor neuron axons. hnRNP-Q is a known component of mRNA transport granules and has been previously shown to be involved in various aspects of mRNA regulation. Here we will use cultured neurons from mouse embryos to test the hypotheses that hnRNP-Q1 is required for mRNA localization and that interactions with SMN can influence this trafficking. These studies will provide new insight into the

pathomechanism involved in SMA and should identify new targets for therapeutic intervention.

Tamara Caspary, Ph.D.

(RG) Defining the Role of Arl13b Interacting Proteins in the Neural-Glial Switch

\$ 148,863 07/01/2009 – 06/30/2010 (Year 1)

\$ 148,422 07/01/2010 - 06/30/2011 (Year 2)

\$ 153,642 07/01/2011 - 06/30/2012 (Year 3)

Summary: In a previous MDA Development Grant we characterized a novel protein, Arl13b, which when absent from the spinal cord leads to too many motor neurons and a lack of their insulating cells, oligodendrocytes. Our work showed that Arl13b links the signaling pathways that specify these cell types to cilia, the projections on cells classically thought to be involved in cell motility. It turns out that there are also immotile cilia, or sensory cilia, present on every type of mammalian cell. The fact that such cells are necessary for signaling, so that cells know their identity, is a new discovery. Here we aim to define this link. We have already identified a protein with which Arl13b interacts. By delineating the nature of this interaction, identifying mutations that interfere with the interaction, and isolating the role of the interacting protein in the developing spinal cord, we aim to define the mechanism through which cilia are needed for normal motor neuron and oligodendrocyte development.

Jonathan Glass, M.D., MD

(RRG) Models and Treatments of Motor Neuron Disease

\$ 36,896 02/01/2011 - 01/31/2012 (Year 1)

Summary: A major question for neurodegenerative diseases, and specifically for ALS, is why some neurons die and others are spared during the disease process. In ALS, motor neurons are affected almost exclusively, manifesting clinically as progressive weakness and pathologically as loss of motor neurons in the brain and spinal cord. And even within motor neuron populations, some are more susceptible than others - i.e. neurons controlling eye movements and sphincters are spared. There are no good explanations for this phenomenon, but understanding selective vulnerability may provide important clues to disease pathogenesis. The mouse model of familial ALS (G93A) also shows this selective vulnerability for motor neurons, and is thus a good model for testing hypotheses. In this project we will use newly generated antibody reagents to identify populations of motor neurons that may be "ready to die", and correlate these findings with other markers of cellular stress.

Grace Pavlath, Ph.D.

(RG) Mechanisms of Myofiber Branching

\$ 98,423 02/01/2011 - 01/31/2012 (Year 1)

\$ 98,423 02/01/2012 - 01/31/2013 (Year 2)

\$ 98,423 02/01/2013 - 01/31/2014 (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.

Wilfried Rossoll, Ph.D.

(RG) The role of the ALS disease protein TDP-43 in motor neurons

\$	119,551	07/01/2010 – 06/30/2011	(Year 1)
\$	119,551	07/01/2011 – 06/30/2012	(Year 2)
\$	119,551	07/01/2012 – 06/30/2013	(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 Health Sciences University Research Institute, Inc.

Lin Mei, M.D./Ph.D.

(RG) Neuromuscular junction formation

\$	130,939	07/01/2009 – 06/30/2010	(Year 1)
\$	130,939	07/01/2010 - 06/30/2011	(Year 2)
\$	130,939	07/01/2011 - 06/30/2012	(Year 3)

Summary: Muscle contraction occurs when the neurotransmitter acetylcholine binds to its receptor (AChR). This receptor needs to be concentrated in a region of muscle fibers where the nerve contacts the muscle for fast and efficient neurotransmission. Improper localization and malfunction of the AChR is implicated in neurological disorders including the congenital myasthenic syndrome and myasthenia gravis. We have recently discovered a missing link – LRP4 – in our understanding of how AChR becomes concentrated at the nerve-muscle contact. We identify LRP4 as a functional coreceptor of agrin that is necessary for agrin-induced MuSK signaling and AChR clustering. Our research is aimed at understanding mechanisms of how the novel protein LRP4 regulates agrin/MuSK signaling and AChR clustering. These studies will contribute to developing strategies of gene therapy and of diagnostic tools for neuromuscular disorders including congenital myasthenic syndrome and myasthenia gravis.

2012 ACTIVE GRANTS

IOWA

Iowa City - The University of Iowa

Kevin Campbell, PhD

(RG) Molecular Basis of Glycosylation-Deficient Muscular Dystrophy

\$ 158,623 01/01/2010 - 12/31/2010 (Year 1)

\$ 158,623 01/01/2011 - 12/31/2011 (Year 2)

\$ 158,623 01/01/2012 - 12/31/2012 (Year 3)

Summary: Our long term goal is to design a therapy for dystroglycan glycosylation-deficient limb-girdle and congenital muscular dystrophy. Many questions remain concerning which genes are involved in dystroglycan modification and what they do during the process. These questions need to be resolved for the development of effective treatment strategies. This project focuses on exploring the mechanisms required for dystroglycan posttranslational processing. The overall results of these experiments will lead to identification and understanding of both genes that promote dystroglycan modification and those that inhibit it. Future studies will use this information for the development of therapeutic strategies to treat glycosylation-deficient muscular dystrophy

Jennifer Levy, Ph.D.

(DG) Pathways and consequences of non-dysferlin mediated membrane repair

\$ 60,000 08/01/2011 - 07/31/2012 (Year 1)

\$ 60,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 60,000 08/01/2013 - 07/31/2014 (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 dysferlin-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 Lueck, Ph.D.

(DG) Pathophysiology of Muscle Weakness and Wasting in Myotonic Dystrophy

\$ 60,000 02/01/2011 - 01/31/2012 (Year 1)

\$ 60,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 60,000 02/01/2013 - 01/31/2014 (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.

2012 ACTIVE GRANTS

ILLINOIS

Chicago - Illinois Institute of Technology

Nick Menhart, Ph.D.

(RG)	Biophysics of Exon Skipped Dystrophin Rods		
\$	93,577	02/01/2012 - 01/31/2013	(Year 1)
\$	85,837	02/01/2013 - 01/31/2014	(Year 2)
\$	85,837	02/01/2014 - 01/31/2015	(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 University of Chicago

Elizabeth McNally, M.D., Ph.D.

(RG)	Ferlin proteins in receptor recycling, membrane repair and muscular dystrophy		
\$	106,796	01/01/2010 - 12/31/2010	(Year 1)
\$	106,796	01/01/2011 - 12/31/2011	(Year 2)
\$	106,796	01/01/2012 - 12/31/2012	(Year 3)

Summary: Mutations in the dysferlin gene cause limb girdle muscular dystrophy. Dysferlin mutations cause dysferlin to be lost from muscle. It has been suggested that dysferlin loss leads to muscular dystrophy by rendering the muscle less able to repair itself when it is damaged, but the means by which abnormal repair occurs is unknown. We studied myoferlin, a protein highly related to dysferlin. We have found that myoferlin regulates the movement of vesicles inside muscle cells. We propose that is the abnormal movement and accumulation of these vesicles that leads to muscle dysfunction. We propose to test whether dysferlin alters the same processes as myoferlin and to determine whether these proteins have overlapping functions. These studies will lead to better tests for dysferlin function that can ultimately be used to test forms of therapy.

Kamal Sharma, Ph.D.

(RG) Genetic analysis of premotor excitatory interneurons in the mouse spinal cord

\$	153,590	01/01/2010 - 12/31/2010	(Year 1)
\$	143,058	01/01/2011 - 12/31/2011	(Year 2)
\$	143,058	01/01/2012 - 12/31/2012	(Year 3)

Summary: Motor neurons are the primary target of pathological changes that result in muscle dysfunction observed in ALS and SMA patients. Although motor neurons are the only neurons that directly deliver neural signals to the muscle, premotor neurons in the spinal cord are critical for determining the timing and strength of the neural signals that motor neurons would generate. Whether genetic mutations in that cause familial ALS or SMA affect the functions and survival of premotor neurons in the spinal cord is not known. Main hurdles in testing the involvement of premotor neurons in motor neuron disease have been the lack of biomarkers for identifying these neurons in the spinal cord, availability of assays to quantify premotor-to-motor neuron input and genetic models to study the function of these neurons in normal and disease state. We have recently identified one class of excitatory, glutamatergic premotor neurons in the mouse spinal cord, called the V2a neurons. We have generated transgenic mouse models to study their connectivity and function. These studies have shown that V2a interneurons provide critical excitatory input to motor neurons for breathing and locomotion. In this project our first goal is to test whether the disease-causing mutations in *sod1* (familial ALS) and *smn1* (SMA) affect the function and survival of V2a premotor neurons. Next we will test whether the absence of V2a-to-motor neuron excitatory drive affects the onset and severity of ALS and SMA.

Chicago - University of Illinois

Jesus Garcia-Martinez, M.D., Ph.D.

(RG) Function of the alpha2/delta1 subunit in dystrophic muscle

\$	108,615	07/01/2009 - 06/30/2010	(Year 1)
\$	111,625	07/01/2010 - 06/30/2011	(Year 2)
\$	114,707	07/01/2011 - 06/30/2012	(Year 3)

Summary: The alpha2/delta1 subunit is a membrane protein that forms part of a calcium channel complex necessary to initiate contraction in adult muscle. However, we recently found new surprising evidence indicating that alpha2/delta1 is not associated with this calcium channel complex in myoblasts and that it has novel and previously unidentified roles. Our newest evidence indicates that the alpha2/delta1 subunit is involved in detecting and transmitting extracellular signals to the interior of muscle precursor cells. Muscle repair is essential for maintaining and improving function in patients with Duchenne muscular dystrophy. Central to the repair mechanism is the activation of satellite cells. The goal of this project is to determine the role of the alpha2/delta1 subunit in the activation and migration of satellite cells in muscle from the mdx mouse model of Duchenne muscular dystrophy.

Matthew Meriglioli, M.D.

(RG) Antigen-specific Immunoregulation of Experimental Myasthenia Gravis

\$	111,597	07/01/2009 - 06/30/2010	(Year 1)
\$	111,962	07/01/2010 - 06/30/2011	(Year 2)

\$ 114,328 07/01/2011 - 06/30/2012 (Year 3)

Summary: Autoimmune myasthenia gravis (MG) is a prototypic autoimmune disorder in which the immune system targets the skeletal muscle acetylcholine receptor (AChR) causing symptoms of muscle weakness. Research in this project will build upon current studies in the applicant's laboratory which focus on the immune regulation in the mouse model of MG. We have shown that treatment with a particular growth factor, GM-CSF, effectively suppresses MG in mice and that this clinical disease suppression is associated with the mobilization of "immune regulatory cells." The current research will attempt to move this strategy closer to application to human MG by performing in vitro studies utilizing immature cells obtained from animals with experimental MG, and determining whether these immune regulatory cells can be grown in culture for later administration as treatment. We will examine the functional properties of these cells and whether their administration leads to suppression of immune reactivity to the AChR and improvement of experimental disease.

Matthew Meriggioli, M.D.

(ACTG) GM-CSF in the therapy of autoimmune myasthenia gravis

\$ 175,974 03/01/2011 - 12/31/2011 (Year 1)

\$ 177,583 01/01/2012 - 12/31/2012 (Year 2)

\$ 176,923 01/01/2013 - 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.

JianRong Sheng, Ph.D

(DG) Immune Regulation of Experimental Autoimmune Myasthenia Gravis

\$ 60,000 01/01/2010 - 12/31/2010 (Year 1)

\$ 60,000 01/01/2011 - 12/31/2011 (Year 2)

\$ 60,000 01/01/2012 - 12/31/2012 (Year 3)

Summary: In myasthenia gravis (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. It appears that this treatment leads to suppression of the immune cells (T cells and 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 in mice. The information gained from these studies may help to develop a better treatment for human MG that harnesses the immune system's own regulatory network to re-establish "immune harmony," eliminating 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 08/01/2011 - 07/31/2012 (Year 1)

\$ 60,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 60,000 08/01/2013 - 07/31/2014 (Year 3)

Summary: 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

Renzhi Han, Ph.D.

(RG) Efficacy of complement inhibition as a therapeutic strategy for dysferlinopathy

\$ 135,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 135,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 135,000 07/01/2012 - 06/30/2013 (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.

2012 ACTIVE GRANTS

INDIANA

Indianapolis - Indiana University

William Groh, M.D.

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

\$ 94,588 01/01/2010 - 12/31/2010 (Year 1)

\$ 90,440 01/01/2011 - 12/31/2011 (Year 2)

\$ 89,751 01/01/2012 - 12/31/2012 (Year 3)

Summary: In 1997 we initiated recruitment into a clinical Registry of Arrhythmias in Myotonic 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.

2012 ACTIVE GRANTS

KENTUCKY

Lexington - University of Kentucky Research Foundation

Stefan Stamm, Ph.D.

(RG)	Promotion of exon 7 inclusion to treat spinal muscular atrophy		
\$	129,828	07/01/2009 – 06/30/2010	(Year 1)
\$	129,828	07/01/2010 - 06/30/2011	(Year 2)
\$	129,828	07/01/2011 - 06/30/2012	(Year 3)

Summary: Spinal muscular atrophy (SMA) is caused by the loss of one gene, SMN1. All patients with SMA have an almost identical 'backup' gene, SMN2. The problem is that the body cannot put a critical piece of genetic information into the readout of the SMN2 gene. As a result, the SMN2 gene cannot substitute the loss of SMN1. However, the program to include this genetic information can be stimulated, for example by drugs like valproic acid that are now in clinical testing. It is not clear how these drugs affect the cellular program, known as alternative splice site selection. This is a problem, because it prevents a rational improvement of therapies. The project aims to understand how this stimulation works and how drugs promote the correct use of the SMN2 gene. We will also test a new class of chemical compounds at hand that would selectively promote the inclusion of the missing critical piece of genetic information.

Louisville - University of Louisville Research Foundation, Inc.

Ashok Kumar, Ph.D.

(RG)	Therapeutic Targeting of Matrix Metalloproteinases in Muscular Dystrophy		
\$	116,402	07/01/2010 - 06/30/2011	(Year 1)
\$	116,402	07/01/2011 - 06/30/2012	(Year 2)
\$	116,402	07/01/2012 - 06/30/2013	(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.

2012 ACTIVE GRANTS

MASSACHUSETTS

Boston - Beth Israel Deaconess Medical Center

Zolt Arany, M.D. Ph.D.

(RG)	PGC-1 coactivators and angiogenesis in muscular dystrophy		
\$	118,232	07/01/2010 - 06/30/2011	(Year 1)
\$	116,978	07/01/2011 - 06/30/2012	(Year 2)
\$	116,978	07/01/2012 - 06/30/2013	(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.

Boston - Boston University

Mahasweta Girgenrath, Ph.D

(RG)	Modulation of Inflammation in the context of Regeneration in MDC1A		
\$	119,183	02/01/2012 - 01/31/2013	(Year 1)
\$	119,133	02/01/2013 - 01/31/2014	(Year 2)
\$	119,149	02/01/2014 - 01/31/2015	(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 associated with muscular dystrophy resulting due to lack of laminin alpha 2, an extracellular matrix protein.

Boston - Brigham and Women's Hospital, Inc.

Steven Greenberg, MD

(RG)	The role of TDP-43 and aberrant RNA metabolism in sIBM and hIBM		
\$	144,314	02/01/2011 - 01/31/2012	(Year 1)

\$ 150,000 02/01/2012 - 01/31/2013 (Year 2)
\$ 150,000 02/01/2013 - 01/31/2014 (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.

Pavel Ivanov, Ph D

(DG) Angiogenin and Amyotrophic Lateral Sclerosis

\$ 60,000 01/01/2010 - 12/31/2010 (Year 1)
\$ 60,000 01/01/2011 - 12/31/2011 (Year 2)
\$ 60,000 01/01/2012 - 12/31/2012 (Year 3)

Summary: Genetic studies have identified several genes that are linked to the development of ALS. One of these genes encodes angiogenin, a ribonuclease that promotes blood vessel development and cell survival. Mutants of angiogenin which lose these biological properties promote ALS in a subset of patients, but the mechanism is not understood. We have discovered that angiogenin is involved in a stress response program that allows cells to survive adverse environmental conditions. This stress response program operates on the post-transcriptional level of gene expression and acts through the formation of a novel class of small non-protein coding RNAs. We hypothesize that mutant angiogenin abrogates the normal function of this stress program and consequently inhibits cell survival. During the first phase of our investigation, we will explore the effect of angiogenin and its ALS-specific mutants using cell lines suitable for studying both stress response and cell survival. Upon successful completion of this part we will apply these results to an investigation using cultured motor neurons.

Calum MacRae, MB.ChB, Ph.D.

(RG) In vivo analysis of myocardial patterning in zebrafish muscular dystrophy models

\$ 91,622 07/01/2009 - 06/30/2010 (Year 1)
\$ 90,270 07/01/2010 - 06/30/2011 (Year 2)
\$ 92,209 07/01/2011 - 06/30/2012 (Year 3)

Summary: One of the most important complications of muscular dystrophy is involvement of the heart, which can lead to irregular heart rhythms, including heart block or sudden death, and reduced pumping strength or congestive heart failure. This cardiac involvement contributes significantly to the burden of disease, and is one of the major causes of death in many forms of muscular dystrophy. The mechanisms by which the heart is affected in muscular dystrophies remain poorly understood. Using the zebrafish, we have developed a unique set of techniques to investigate the ways in which genetic abnormalities cause various forms of heart disease. The zebrafish is transparent and so enables the study of heart abnormalities as they actually occur early in heart formation in genetically faithful models of some forms of muscular dystrophy. This zebrafish approach is very powerful since once we better understand the mechanisms of the heart disease in muscular dystrophy, we can adapt the zebrafish models of muscular dystrophy we have created to detect the heart involvement using

automated microscopes. This automation will then allow us to test many thousands of chemical compounds in order to identify specific chemicals that reduce or reverse the muscular dystrophy heart defects in the zebrafish. This will rapidly identify potential new drugs for the treatment of muscular dystrophy.

Xin Wang, Ph.D.

(RG) Screening Agonists of the Melatonin Receptor 1A against ALS

\$ 143,877 01/01/2010 - 12/31/2010 (Year 1)

\$ 143,877 01/01/2011 - 12/31/2011 (Year 2)

\$ 143,877 01/01/2012 - 12/31/2012 (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.

Boston - Children's Hospital Boston

Alan Beggs, Ph.D.

(RG) Molecular Genetics of Congenital Myopathies

\$ 133,978 08/01/2011 - 07/31/2012 (Year 1)

\$ 133,978 08/01/2012 - 07/31/2013 (Year 2)

\$ 129,034 08/01/2013 - 07/31/2014 (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.

Steven Greenberg, MD

(RG) Development and Screening of Cellular Models of Dermatomyositis

\$	112,856	01/01/2010 - 12/31/2010	(Year 1)
\$	112,247	01/01/2011 - 12/31/2011	(Year 2)
\$	113,166	01/01/2012 - 12/31/2012	(Year 3)

Summary: Dermatomyositis is an autoimmune neuromuscular disease that appears to be driven by the overproduction of type 1 interferons. Current therapeutic development of anti-interferon alpha therapy is underway in a myositis clinical trial. I will develop cellular models of muscle injury representing a process occurring in dermatomyositis in order to screen these models to learn the most basic mechanisms by which type 1 interferons injure muscle, and to identify compounds that prevent such injury.

Emanuela Gussoni, Ph.D

(RG) Melanoma cell adhesion molecule (MCAM) in human myogenic cells

\$	128,022	08/01/2011 - 07/31/2012	(Year 1)
\$	128,022	08/01/2012 - 07/31/2013	(Year 2)
\$	128,022	08/01/2013 - 07/31/2014	(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 Kang, M.D.

(RG) Linkage analysis in limb-girdle muscular dystrophy

\$	99,870	02/01/2011 - 01/31/2012	(Year 1)
\$	99,938	02/01/2012 - 01/31/2013	(Year 2)
\$	99,914	02/01/2013 - 01/31/2014	(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	07/01/2010 - 06/30/2011	(Year 1)
\$	125,000	07/01/2011 - 06/30/2012	(Year 2)
\$	125,000	07/01/2012 - 06/30/2013	(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.

Fedik Rahimov, Ph.D.

(DG)	Biomarker Discovery in Muscles from FSHD Patients		
\$	60,000	08/01/2011 - 07/31/2012	(Year 1)
\$	60,000	08/01/2012 - 07/31/2013	(Year 2)
\$	60,000	08/01/2013 - 07/31/2014	(Year 3)

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	08/01/2011 - 07/31/2012	(Year 1)

\$ 101,443 08/01/2012 - 07/31/2013 (Year 2)
\$ 101,443 08/01/2013 - 07/31/2014 (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-1 α rescues some of the defective mitochondrial function both in cell culture and mouse models of mitochondrial myopathies. Importantly, activation of PGC-1 α function prevented an energetic deficit and effectively improve the mitochondrial myopathy phenotype in mouse models. Thus, activation of PGC-1 α , or components of its molecular pathway, could be a treatment for mitochondrial myopathy patients. However, because PGC-1 α 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-1 α pathway is activated, here we propose two new strategies. First, activation of PGC-1 α through the mTOR/YY1 complex and, second, small molecule chemical screening to identify compounds that activate PGC-1 α using cybrid cell lines carrying genetic mutations from mitochondrial myopathy patients.

Boston - Gordon Research Conferences

P D Allen, M.D., Ph.D.

(SGNEW) 2012 Excitation Coupling Gordon Conference
\$ 10,000 06/01/2012 – 06/30/2012 (Year 1)

Summary: The Gordon Research Conference on Excitation Contraction Coupling (ECC) is the only extended meeting that focuses on the physiological, biochemical, biophysical, structural and molecular aspects of ECC and on how ECC is altered by, and/or contributes to muscle disease. No comparable conference on this scale has been organized since 2009, or is planned in the near future. It will bring together a diverse group of experts from all over the world together with junior investigators and trainees, in a comfortable setting that will maximize interactions among conference attendees.

Boston - Harvard Medical School

Alfred Goldberg, Ph.D.

(RG) Protein breakdown in muscle in normal and disease states
\$ 139,979 08/01/2011 - 07/31/2012 (Year 1)
\$ 135,399 08/01/2012 - 07/31/2013 (Year 2)
\$ 135,399 08/01/2013 - 07/31/2014 (Year 3)

Summary: 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.

Andrew Lassar, Ph.D.

(RG) Regulation of quiescence and differentiation of satellite cells.

\$	125,000	01/01/2010 - 12/31/2010	(Year 1)
\$	125,000	01/01/2011 - 12/31/2011	(Year 2)
\$	125,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: Satellite cells comprise a skeletal muscle stem cell population that must remain quiescent in an undifferentiated state and capable of future cell division. Despite the requirement for quiescent satellite cells to remain undifferentiated, these cells are known to express the transcription factor, Pax7, a crucial regulator of satellite induction, maintenance and differentiation. While it is known that Pax7 can induce the expression of MyoD, the ability of Pax7 to induce MyoD must be constrained in quiescent satellite cells, as MyoD expression is absent from quiescent satellite cells and specifically induced in activated satellite cells. I will elucidate how BMP signals block the ability of Pax7 to induce MyoD expression and test whether such signals indeed block premature expression of MyoD in skeletal muscle progenitor cells (in the embryo) or in quiescent satellite cells (in the adult). In addition, I will determine whether Id2 and Id3 are necessary to block differentiation of quiescent satellite cells.

Edward Owusu-Ansah, Ph.D

(DG) A Molecular Genetic Analysis of Mitochondrial Myopathy

\$	60,000	08/01/2011 - 07/31/2012	(Year 1)
\$	60,000	08/01/2012 - 07/31/2013	(Year 2)
\$	60,000	08/01/2013 - 07/31/2014	(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 - Massachusetts General Hospital

Andrew Brack, PhD

(RG) Maintenance of the satellite cell pool in murine dystrophic muscle

\$ 117,857 02/01/2011 - 01/31/2012 (Year 1)

\$ 117,701 02/01/2012 - 01/31/2013 (Year 2)

\$ 117,701 02/01/2013 - 01/31/2014 (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 Cudkowicz, M.D., MD

(RG) Trial of high fat/high calorie diet versus optimal calorie replacement in ALS

\$ 231,766 07/01/2009 – 06/30/2010 (Year 1)

\$ 218,525 07/01/2010 – 06/30/2012 (Year 2)

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.

Cambridge - Achelois Biosciences

Jasbir Sehra,

(RRG) Restricted Funds for Research - McCaw Fund

\$ 39,192 11/1/2011 – 10/31/2012 (Year 1)

Cambridge - ALS Therapy Development Foundation Inc.

Steven Perrin, Ph.D.

(MVP) Ctl4_Fc: Immunomodulation of Costimulatory Signaling as a Therapeutic Strategy for ALS

\$	86338	-	(Year 1)
\$	45837	-	(Year 2)
\$	146675	-	(Year 3)

Summary:

Cambridge - Harvard College

Alexander Schier, Ph.D.

(RG) Cilia in muscle and motoneuron development

\$	130,040	01/01/2010 - 12/31/2010	(Year 1)
\$	130,040	01/01/2011 - 12/31/2011	(Year 2)
\$	130,040	01/01/2012 - 12/31/2012	(Year 3)

Summary: The primary cilium, a hair-like structure present on the surface of most vertebrate cells, performs not only sensory functions but is also involved in several signal transduction pathways. Despite the presence of primary cilia on muscle cells, their functions in muscle development and regeneration remain largely unknown. We recently discovered that elimination of all primary cilia in zebrafish results in defects in muscle formation and muscle innervation. Our goal is to determine how primary cilia affect neuromuscular generation, maintenance and degeneration, and to identify drugs that can ameliorate the disease.

Charlestown - Massachusetts General Hospital

Nazem Atassi, MD.

(CRTG) Comparative effectiveness in ALS symptomatic management

\$	90,000	07/01/2010 - 06/30/2011	(Year 1)
\$	90,000	07/01/2011 - 06/30/2012	(Year 2)

Summary: We are proposing this study to determine if people with ALS can tolerate Tizanidine treatment for muscle stiffness. The long term goal is to determine the best management for a common symptom of ALS. Amyotrophic Lateral Sclerosis (ALS) is a disorder affecting the nervous system. In addition to muscle weakness, people with ALS suffer from symptoms such as stiffness, cramps, breath or swallowing difficulties, increased saliva, and depression. Potential treatments for most of these symptoms are available. The challenge is that many of these treatments were developed for people who don't have ALS. As a result, we don't know if these treatments are tolerated, safe, or effective in people with ALS. Treatment of ALS symptoms is very important. For example, the recent use of an assisting breathing machine by people with ALS improved their sleeping, quality of life and prolonged their survival. Muscle stiffness is a common problem in ALS that usually causes slowing of movements and loss of coordination in the arms and legs. As a result ambulation and usual daily activities become difficult and often painful. We don't know the ideal dose or the effect of any of the anti-stiffness medication in ALS. Tizanidine is an approved medication that reduces muscle stiffness in other diseases. We designed this study to determine the range of dosages that people with ALS can tolerate and to obtain information about the safety and effectiveness of Tizanidine in treating muscle stiffness in ALS.

James Berry, M.D., M.P.H.

(CRTG) A Phase I/II Study of a Continuous Infusion of ISIS 333611 in SOD1 Familial ALS

\$	90,000	07/01/2011 - 06/30/2012	(Year 1)
\$	90,000	07/01/2012 - 06/30/2013	(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.

Everett - 4S3 BIOSCIENCE INC

Dustin Armstrong, Ph.D.

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

\$	148,138	04/01/2010 - 03/31/2011	(Year 1)
\$	112,153	04/01/2011 - 03/31/2012	(Year 2)

Summary: Myotubular myopathy (MTM) is a rare and severe sex-linked muscle disease that occurs in 1 male in every 50,000 births and is caused by a lack of the myotubularin 1 (MTM1) protein. At birth MTM patients present with muscle weakness and respiratory difficulties and those that survive the neonatal period are often totally or partially dependent upon the support of a ventilator. Patients with MTM exhibit delayed motor development and are susceptible to numerous medical complications. The average hospital stay for neonatal MTM patients is ~90 days, and the need for long-term ventilatory assistance and in-home care, as well as the costs associated with medical complications impose a substantial personal and economic burden to patients and families. Therapies for MTM clearly need to be developed, and we will test whether a muscle-targeted myotubularin 1 can alleviate the disease in the mouse model of human MTM. Successful conclusion of this study will be followed by production and clinic

Watertown - Boston Biomedical Research Institute

Charles Emerson, Ph.D.

(RG) Evaluating DUX4 as a therapeutic target for FSHD

\$	200,000	02/01/2012 - 01/31/2013	(Year 1)
\$	200,000	02/01/2013 - 01/31/2014	(Year 2)

\$ 200,000 02/01/2014 - 01/31/2015 (Year 3)

Summary: Facioscapulohumeral muscular dystrophy (FHD) 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 FHD. 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 FHD. 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 FHD therapeutics. We expect that the results of our studies will provide critical information needed to develop effective and specific DUX4-targeted therapies for FHD.

Jeffrey Miller, PhD

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

\$ 114,620 02/01/2012 - 01/31/2013 (Year 1)

\$ 114,620 02/01/2013 - 01/31/2014 (Year 2)

\$ 114,620 02/01/2014 - 01/31/2015 (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.

Worcester - University of Massachusetts Medical School

Michael Francis, PhD

(RG) Genetic analysis of a *C. elegans* model of slow-channel myasthenic syndrome

\$ 110,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 110,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 110,000 07/01/2012 - 06/30/2013 (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.

2012 ACTIVE GRANTS

MARYLAND

Baltimore - Hugo W. Moser Research Institute at Kennedy Krieger, Inc.

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

(RG) Myostatin Regulates Fate of Satellite Cells in Dystrophic Muscle

\$ 117,513 08/01/2011 - 07/31/2012 (Year 1)

\$ 117,513 08/01/2012 - 07/31/2013 (Year 2)

\$ 117,513 08/01/2013 - 07/31/2014 (Year 3)

Summary: In most muscular dystrophies and chronic myopathies, muscle regeneration becomes less effective over time and muscle is replaced 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.

Baltimore - Johns Hopkins University School of Medicine

Elizabeth Chen, Ph.D.

(RG) Functional analysis of the small GTPase Rho1 in myoblast fusion in vivo

\$ 107,163 02/01/2012 - 01/31/2013 (Year 1)

\$ 107,163 02/01/2013 - 01/31/2014 (Year 2)

\$ 107,163 02/01/2014 - 01/31/2015 (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 Farah, PhD

(RG) New strategies for Axonal Protection in Murine Models of CMT type 1

\$	150,240	01/01/2010 - 12/31/2010	(Year 1)
\$	150,240	01/01/2011 - 12/31/2011	(Year 2)
\$	150,240	01/01/2012 - 12/31/2012	(Year 3)

Summary: This project will test a new hypothesis for the pathogenesis of the axonal degeneration that underlies the clinical manifestations of the demyelinating forms of Charcot-Marie-Tooth disease, and will test in animal models novel therapeutic approaches. Importantly, if successful this therapy could move rapidly to human trials. The primary therapeutic agents that we will test are, for very different reasons, under active development by pharmaceutical companies interested in Alzheimer's disease and multiple sclerosis.

David Kass, MD

(RG) Protein Kinase G inhibition of TRPC to Benefit the Dystrophin-deficient Heart

\$	105,309	02/01/2011 - 01/31/2012	(Year 1)
\$	105,309	02/01/2012 - 01/31/2013	(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	08/01/2011 - 07/31/2012	(Year 1)
\$	59,999	08/01/2012 - 07/31/2013	(Year 2)
\$	59,999	08/01/2013 - 07/31/2014	(Year 3)

Summary: 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.

Brett Morrison, M.D., Ph.D.

(RG) Astroglial Monocarboxylate Transporter (MCT) Pathway in ALS

\$	100,001	01/01/2010 - 12/31/2010	(Year 1)
\$	100,001	01/01/2011 - 12/31/2011	(Year 2)
\$	100,001	01/01/2012 - 12/31/2012	(Year 3)

Summary: There is a growing body of data implicating astroglial dysfunction as a significant contributor to the pathogenesis of amyotrophic lateral sclerosis (ALS), but the specific cellular defects are not fully understood. We will study a novel mechanism for neurodegeneration in ALS, impairment of astroglial monocarboxylate transporters (MCTs), which may be a significant contributor to disease progression. If successful, we expect this research to lead to the production of novel treatments for ALS, designed to reverse the impairment in MCTs.

Thien Nguyen, M.D., Ph.D.

(RG) Axonal protection in Demyelinating Disorders

\$	140,000	02/01/2011 - 01/31/2012	(Year 1)
\$	140,000	02/01/2012 - 01/31/2013	(Year 2)
\$	140,000	02/01/2013 - 01/31/2014	(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 Rothstein, M.D., Ph.D.

(RG) Small Molecule Induced Astroglialgenesis

\$	123,095	01/01/2010 - 12/31/2010	(Year 1)
\$	123,095	01/01/2011 - 12/31/2011	(Year 2)

\$ 123,095 01/01/2012 - 12/31/2012 (Year 3)

Summary: This project is focused on the use of relevant rodent and human progenitor cells as a discovery tool to identify drugs that may transdifferentiate existing adult glial progenitor cells and lead to the generation of new glial cells. The generation of new astroglial cells could be therapeutically valuable for neurological diseases including ALS. Most importantly, it could generate adult stem cell therapy, by activating endogenous adult CNS progenitor cells to differentiate into new astroglia, thereby eliminating need for external cellular based therapy.

Shanthini Sockanathan, PhD

(RG) Characterization of a new animal model for motor neuron degeneration

\$ 121,026 07/01/2010 - 06/30/2011 (Year 1)

\$ 112,942 07/01/2011 - 06/30/2012 (Year 2)

\$ 113,864 07/01/2012 - 06/30/2013 (Year 3)

Summary: In order to prevent or cure motor neuron degenerative diseases, there is a pressing 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 02/01/2011 - 01/31/2012 (Year 1)

\$ 132,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 132,000 02/01/2013 - 01/31/2014 (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 (GDPE) 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.

Jiou Wang, M.D., Ph.D.

(RG) Elucidating the Molecular Mechanisms of ALS in *C. elegans* and Mammalian Models

\$ 110,000 02/01/2011 - 01/31/2012 (Year 1)

\$ 110,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 110,000 02/01/2013 - 01/31/2014 (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

Robert Bloch, Ph.D.

(RG) Cellular and Molecular Studies of Dysferlinopathy

\$ 120,765 02/01/2012 - 01/31/2013 (Year 1)

\$ 120,765 02/01/2013 - 01/31/2014 (Year 2)

\$ 120,765 02/01/2014 - 01/31/2015 (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.

Rockville - Food and Drug Administration

Jakob Reiser, PhD

(RG)	Remote therapeutic gene delivery for SMA		
\$	80,837	09/01/2009 – 08/31/2010	(Year 1)
\$	87,504	09/01/2010 – 08/31/2011	(Year 2)
\$	81,771	09/01/2011 – 08/31/2012	(Year 3)

Summary: This study seeks to identify a practical means of gene therapy in Spinal Muscular Atrophy (SMA). SMA, the leading inherited cause of death in children under two years of age, results from the progressive loss of lower motor neurons. While the disease lacks any validated treatments, recent advances confirm that loss of function in one of the survival of motor neuron genes (SMN1) causes SMA. The availability of defined genetic (SMN gene) and anatomical (motor neurons) targets has prompted enthusiasm for rapid translation of experimental therapies for this disease. SMA has also been recognized as a model for the development of translational therapies in a wide range of neurodegenerative disease. Finally, the identification of SMN loss of function as the key cause of SMA has focused attention on gene transfer as an optimal approach to this disorder.

2012 ACTIVE GRANTS

MICHIGAN

Ann Arbor - University of Michigan

James Dowling, M.D., Ph.D.

(RG) Neuromuscular junction transmission and myotubular myopathy

\$ 117,893 02/01/2011 - 01/31/2012 (Year 1)

\$ 117,893 02/01/2012 - 01/31/2013 (Year 2)

\$ 117,893 02/01/2013 - 01/31/2014 (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.

John Kim, Ph.D.

(RG) The Characterization of all 3'UTR Isoforms for the Muscle Transcriptome

\$ 131,934 07/01/2009 - 06/30/2010 (Year 1)

\$ 128,919 07/01/2010 - 06/30/2011 (Year 2)

\$ 127,378 07/01/2011 - 06/30/2012 (Year 3)

Summary: Accurate control of gene expression is critical for muscle function. Recently, studies have shown that the 3' untranslated region (the part of genes that follows the coding region but is not "translated" into protein) plays a critical role in the regulation and expression of genes, including genes that are involved in muscle development and function. However, comparatively little is known about the lengths and identities of these 3'UTR regions of genes, although we do know they are highly varied and dynamic. Furthermore, the 3'UTR of genes are critical targets for a new class of genes termed microRNAs. Estimates indicate that microRNAs may regulate as much as half of all genes in an organism, including humans, by binding to the 3'UTRs of the target genes. We believe that in order to get a more complete understanding of how the expression of genes in the muscle is regulated, it is essential that we begin to define systematically the identities of the 3'UTR sequences of all genes that are expressed in the muscle: only when we know what the "normal" sequences for the 3'UTRs are can we identify what is abnormal or mutated in disease states. To that end, we propose to use the latest sequencing technology to map the 3'UTRs of all genes that are expressed in the muscle.

We believe that this study will help define the reference set of 3'UTRs for muscle genes and will help elucidate the sequence elements in the 3'UTR that are important in the regulation of muscle gene expression.

East Lansing - Michigan State University

William Atchison, Ph.D.

(RG) Compensatory mechanisms in acetylcholine release in Lambert-Eaton Syndrome

\$ 103,685 02/01/2011 - 01/31/2012 (Year 1)

\$ 102,909 02/01/2012 - 01/31/2013 (Year 2)

\$ 102,909 02/01/2013 - 01/31/2014 (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.

2012 ACTIVE GRANTS

MINNESOTA

Minneapolis - University of Minnesota - Twin Cities

James Ervasti, Ph.D.

(RG) Biophysical Optimization of Therapeutic Dystrophin Constructs

\$ 130,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 130,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 130,000 02/01/2014 - 01/31/2015 (Year 3)

Summary: Duchenne muscular dystrophy is a deadly and common childhood disease caused by mutations that disrupt the expression of the protein dystrophin. Although many therapeutic approaches 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 08/01/2011 - 07/31/2012 (Year 1)

\$ 125,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 125,000 08/01/2013 - 07/31/2014 (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.

David Thomas, Ph.D.

(RG) Muscular dystrophy therapy based on small-molecule activators of Ca²⁺ transport

\$ 130,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 130,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 130,000 02/01/2014 - 01/31/2015 (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 Engel, M.D.

(RG) Congenital Myasthenic Syndromes

\$	122,763	01/01/2010 - 12/31/2010	(Year 1)
\$	122,763	01/01/2011 - 12/31/2011	(Year 2)
\$	122,763	01/01/2012 - 12/31/2012	(Year 3)

Summary: Congenital myasthenic syndromes (CMS) arise from defects in proteins at the nerve-muscle junction. They frequently go undiagnosed or misdiagnosed yet their consequences are often highly disabling. The CMS will be studied by a multifaceted approach that will improve their diagnosis, treatment, and prevention.

2012 ACTIVE GRANTS

MISSOURI

Columbia - University of Missouri

Dawn Cornelison, Ph.D.

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

\$ 80,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 80,000 07/01/2011 - 06/30/2012 (Year 2)

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 02/01/2011 - 01/31/2012 (Year 1)

\$ 175,890 02/01/2012 - 01/31/2013 (Year 2)

\$ 175,890 02/01/2013 - 01/31/2014 (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	02/01/2012 - 01/31/2013	(Year 1)
\$	127,194	02/01/2013 - 01/31/2014	(Year 2)
\$	127,194	02/01/2014 - 01/31/2015	(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.

Christian Lorson, PhD

(RG) The role of enhanced muscle in SMA

\$	120,758	01/01/2009 - 12/31/2009	(Year 1)
\$	117,475	01/01/2010 - 12/31/2010	(Year 2)
\$	120,325	01/01/2011 - 03/31/2012	(Year 3)

Summary: Spinal Muscular Atrophy is the leading genetic cause of infantile death, yet there currently is no treatment or cure. This proposal is designed to test a muscle-enhancing compound and a SMN-inducing compound in a mouse model of disease. These results have the potential to identify novel therapeutic avenues for SMA patients.

Saint Louis - Washington University in St. Louis

Anne Connolly, M.D.

(RG) Clinical Outcome validation in Non-ambulatory and young Boys/Men with DMD

\$	250,269	01/01/2010 - 12/31/2010	(Year 1)
\$	197,688	01/01/2011 - 12/31/2011	(Year 2)
\$	203,080	01/01/2012 - 12/31/2012	(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 than 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.

Marc Diamond, M.D.

(RG) Propagation of Pathology in Neurodegeneration

\$	150,000	01/01/2010 - 12/31/2010	(Year 1)
\$	150,000	01/01/2011 - 12/31/2011	(Year 2)
\$	150,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: This project concerns the molecular mechanisms that underlie neurodegenerative diseases such as amyotrophic lateral sclerosis and other forms of degeneration. The project tests a new idea about how neurodegeneration might spread within the nervous system, which holds that protein aggregates may transfer between cells and thus spread cellular dysfunction in a domino-like effect. The project takes two approaches to the problem. In the first case, it uses experiments in cultured cells to determine mechanisms by which protein aggregates might move between cells. In the second case, it will use experimental animals to test whether the movement of protein aggregates occurs in vivo, and is responsible for neurodegeneration in this context. If the project is successful, it will define completely new mechanisms of neurodegeneration that could have important implications for how we approach the development of therapy.

Conrad Weihl, M.D., Ph.D.

(RG) Autophagic treatment paradigms for desminopathies

\$	132,916	02/01/2012 - 01/31/2013	(Year 1)
\$	132,074	02/01/2013 - 01/31/2014	(Year 2)
\$	132,074	02/01/2014 - 01/31/2015	(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), α B-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 α B-crystallin knockin mouse model. This mouse expresses mutant R120G α B-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 α B-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.

2012 ACTIVE GRANTS

NORTH CAROLINA

Charlotte - Carolinas Medical Center

Susan Sparks, M.D., Ph.D.

(RRG) Longitudinal Assessment and Genetic Understanding of Limb-Girdle Musc. Dystrophy
\$ 172,715 09/01/2011 – 08/31/2012 (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 improve patient understanding of the inheritance and family member risk of developing LGMD.

Xiaohua Wu, PhD, MD

(RG) Enhancing Laminin Binding to Treat Muscular Dystrophies
\$ 118,847 02/01/2011 - 01/31/2012 (Year 1)
\$ 106,015 02/01/2012 - 01/31/2013 (Year 2)

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 Medical Center

Michael Hauser, Ph.D

(RG) The Genetic Basis of Autosomal Dominant LGMD
\$ 127,952 02/01/2011 - 01/31/2012 (Year 1)
\$ 127,952 02/01/2012 - 01/31/2013 (Year 2)

\$ 127,952 02/01/2013 - 01/31/2014 (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.

Paul Rosenberg, M.D.

(RG) The role of STIM1 in muscle disease and performance

\$ 135,911 01/01/2010 - 12/31/2010 (Year 1)

\$ 135,911 01/01/2011 - 12/31/2011 (Year 2)

\$ 135,911 01/01/2012 - 12/31/2012 (Year 3)

Summary: Reduced muscle performance and pathologic changes develop in skeletal myopathies as a result of disturbances in calcium handling. Thus, maintenance of the main calcium store is essential for skeletal muscle function. My lab previously identified STIM1 as a critical regulator of this calcium store. STIM1 is a protein that resides in the membrane of the main calcium storage organelle-sarcoplasmic/endoplasmic reticulum (SR/ER). Mice generated in my lab that lack STIM1 display a severe skeletal muscle myopathy. We aim to further understand the details of the STIM1 mediated calcium entry and how its absence influence muscle function. This work has important implications for novel therapeutic strategies for skeletal myopathies and atrophy.

Winston-Salem - Wake Forest University Health Sciences

Martin Childers, Ph.D.

(ACTG) Gene therapy in canine myotubular myopathy

\$ 120,755 04/01/2011 – 03/31/2012 (Year 1)

\$ 122,123 04/01/2012 – 03/31/2013 (Year 2)

\$ 126,487 04/01/2013 – 03/31/2014 (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-in-human studies.

Martin Childers, Ph.D.

(RG) Dystrophin-deficient cardiomyocytes for high-thruput drug screening

\$	160,000	08/01/2011 - 07/31/2012	(Year 1)
\$	160,000	08/01/2012 - 07/31/2013	(Year 2)
\$	160,000	08/01/2013 - 07/31/2014	(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.

2012 ACTIVE GRANTS

NEW JERSEY

Cranbury - Amicus Therapeutics, Inc.

Eric Sjoberg, Ph.D

(RG) Can Pharmacological Chaperones Inhibit rhGAA ERT Induced Immunogenicity

\$ 111,256 02/01/2012 – 02/01/2013 (Year 1)

\$ 75,116 02/01/2013 – 02/01/2014 (Year 2)

Summary: Enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase (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.

Newark - UMDNJ-New Jersey Medical School

Diego Fraidenraich, Ph.D.

(RG) Pluripotent stem cell-induced corrections in muscle and fat of mdx mice

\$ 125,000 08/01/2011 - 07/31/2012 (Year 1)

\$ 125,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 125,000 08/01/2013 - 07/31/2014 (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.

2012 ACTIVE GRANTS

NEW MEXICO

Albuquerque - University of New Mexico

Richard Cripps, D. Phil.

(RG) A Drosophila model for mammalian muscular dystrophy

\$ 113,187 07/01/2010 - 06/30/2011 (Year 1)

\$ 113,187 07/01/2011 - 06/30/2012 (Year 2)

\$ 113,187 07/01/2012 - 06/30/2013 (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.

2012 ACTIVE GRANTS

NEW YORK

Albany - Research Foundation of SUNY - University at Albany

Li Niu, Ph.D.

(RG)	Making Stable Aptamers as ALS Drug Candidates		
\$	136,872	07/01/2009 – 06/30/2010	(Year 1)
\$	165,792	07/01/2010 - 06/30/2011	(Year 2)
\$	102,572	07/01/2011 - 06/30/2012	(Year 3)

Summary: Excessive activation of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtype of ionotropic glutamate receptors has been hypothesized as one of the leading pathogenic mechanisms for amyotrophic lateral sclerosis (ALS). Developing powerful inhibitors to control the excessive receptor activity is thus a logical therapeutic strategy. In a proof of concept experiment, we have successfully identified a class of AMPA receptor-selective RNA aptamers or RNA inhibitors. The potency of one aptamer rivals any existing AMPA receptor inhibitors ever reported thus far. Unmodified, however, these RNA aptamers are limited in therapeutic applications in vivo by their inherent sensitivity towards ribonucleases, the enzymes that catalyze the degradation of RNA into smaller pieces so that the biological function of the RNA as inhibitors is lost. However, chemical modifications of RNA molecules can turn them into ribonuclease-resistant or biostable aptamers. Thus, making ribonuclease-resistant aptamers is required, as the first step, to translate these aptamers from powerful AMPA receptor antagonists into clinically useful drugs. The specific goal of this proposal is to develop high-affinity, chemically modified aptamers. These chemically modified aptamers will be immediately suitable for testing their neuroprotective effectiveness in ALS cellular and animal models.

Brooklyn - SUNY Downstate Medical Center

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

(RG)	Mechanisms of CNS Disease in X-Linked CMT		
\$	141,415	02/01/2012 - 01/31/2013	(Year 1)
\$	138,608	02/01/2013 - 01/31/2014	(Year 2)
\$	134,764	02/01/2014 - 01/31/2015	(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	02/01/2012 - 01/31/2013	(Year 1)
\$	60,000	02/01/2013 - 01/31/2014	(Year 2)
\$	60,000	02/01/2014 - 01/31/2015	(Year 3)

Summary: SMA is caused by mutations in the SMN1 gene. A closely related SMN2 gene expresses 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.

New York - Columbia University

Hasan Akman, PhD

(DG) Generation and Characterization of a Mouse Model of Polyglucosan Body Disease

\$	58,979	07/01/2008 – 06/30/2009	(Year 1)
\$	58,039	07/01/2009 – 06/30/2010	(Year 2)
\$	59,246	07/01/2010 – 02/28/2012	(Year 3)

Summary: The synthesis of glycogen is catalyzed by the sequential actions of two enzymes: (i) glycogen synthetase, which "strings" glucose to form linear chains to a length of approximately 10 glucose "beads"; and (ii) the branching enzyme, which attaches a short branch of approximately 4 glucose units to a linear chain in an alpha-1,6-glucosidic bond. Glycogen storage disease (GSD) type IV (OMIM 232500) is an autosomal recessive disorder caused by deficiency of the glycogen branching enzyme (GBE), and leads to the accumulation of an abnormal amylopectin-like polysaccharide in multiple tissues, including liver, heart, skeletal muscles, and central nervous system (CNS). A late-onset clinical variant, known as adult polyglucosan body disease (APBD), causes a neurodegenerative disorder simulating amyotrophic lateral sclerosis (ALS, Lou Gehrig disease), but often associated with bladder dysfunction and dementia. The

mutated gene, GBE1, has been mapped to chromosome 3p14. The two naturally occurring animal models of this disorder, American quarter horses and Norwegian forest cats, are not practical laboratory animals. Therefore, we propose to develop a mouse model of GSD IV, which would be invaluable to better understand the pathogenesis of the disease and to test therapeutic strategies aimed at increasing the residual activity of branching enzyme in tissues.

Tomoyuki Awano, Ph.D

(DG) Investigating the existence and role of genetic modifiers of SMA in model mice

\$ 60,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 60,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 60,000 02/01/2014 - 01/31/2015 (Year 3)

Summary: 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

Salvatore DiMauro, M.D.

(RG) Therapeutic strategies in neutral lipid storage disease with myopathy

\$ 136,487 02/01/2012 - 01/31/2013 (Year 1)

\$ 136,487 02/01/2013 - 01/31/2014 (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.

Michio Hirano, M.D.

(RG) Molecular pathogenesis of scapulo-peroneal myopathy due to FHL1 mutations

\$ 136,170 01/01/2009 - 12/31/2009 (Year 1)

\$ 111,870 01/01/2010 - 12/31/2010 (Year 2)

\$ 111,905 01/01/2011 - 02/29/2012 (Year 3)

Summary: Scapulo-peroneal (SP) myopathy (sometimes called scapulo-peroneal muscular dystrophy) is an inherited condition characterized by weakness initially in the upper back (scapula) and lower leg (peroneal) region. We have been studying a large Italian-American family with SP myopathy and have identified the causative mutation in a gene called FHL1. Our goal is to understand how mutations in FHL1 cause degeneration of muscle. Through a careful analysis of defects of FHL1, we hope to develop a rational therapy for SP myopathy and expand our understanding of normal muscle function.

Oliver Hobert, Ph.D.

(RG) Deciphering the function of the ALS gene Tdp-43 using the C.elegans model system

\$ 124,837 07/01/2010 - 06/30/2011 (Year 1)

\$ 124,837 07/01/2011 - 06/30/2012 (Year 2)

\$ 124,837 07/01/2012 - 06/30/2013 (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.

Hiroshi Mitsumoto, MD

(RG) Clinically Meaningful Changes (CMC) Scale in the MDA/ALS Clinical Network

\$ 139,015 07/01/2009 - 06/30/2010 (Year 1)

\$ 121,607 07/01/2010 - 06/30/2011 (Year 2)

\$ 140,160 07/01/2011 - 06/30/2012 (Year 3)

Summary: In this project, we will develop a Clinically Meaningful Changes (CMC) Scale in patients and caregivers. It will assess the overall judgment as to how much medication or other treatments improve the disease in patients' and caregiver's perception. Using the newly approved 5 MDA Clinical Research Network Centers, we will study a total of 100 pairs of patients and caregivers. In Aim 1, we will study the reliability of new scales, including a) a scale asking overall impression of the changes, b) a scale for a subject attaining a goal they realistically want to see, c) retrospective analysis of health states based on patient report, and d) "trade-offs" analyses of treatment benefits versus side effects. We will analyze how reliable they are if we interview by person or by telephone. We will confirm that the two interview methods would be equally reliable. In Aim 2, we will measure how many changes occur in these scales over 6 months. We will compare changes in these scales and ALSFRS-R, the standard scale for ALS disease progression. Using a statistical analysis, we will find the best combination of scales which we would call the final CMC scale. In Aim 3, we will

test the usefulness of the CMC scale in a real clinical trial. In Aim 4, we will also study the clinical meaning of the ALSFRS-R in relation to the level of disability and use of medical treatment and also study how much impact a unit change in ALSFRS-R causes in patients with ALS.

Hiroshi Mitsumoto, MD

(RRG) 2011-2012 Wings Four Independent Proposals

\$ 167,542 09/01/2011 – 08/31/2013 (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 cells outside of the nervous system would provide a clue for the pathogenesis 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.

Umrao Monani, Ph.D.

(RG) Investigating the temporal requirements of the SMN protein in SMA

\$ 112,370 01/01/2010 - 12/31/2010 (Year 1)

\$ 112,370 01/01/2011 - 12/31/2011 (Year 2)

\$ 112,370 01/01/2012 - 12/31/2012 (Year 3)

Summary: Of critical importance to the development of an effective treatment of a disease is to understand the temporal requirements of the protein involved and to determine the effects it has during pre- as well as post-natal development. Here, we will use mouse models of human Spinal Muscular Atrophy (SMA) to answer these questions about the SMN protein. Our investigation constitutes a vital aspect of pre-clinical development of a treatment for SMA and our results are expected to contribute to a safe and effective therapy for the disease.

Livio Pellizzoni, Ph.D.

(RG) Regulation of SMN function in ribonucleoprotein assembly

\$ 127,520 07/01/2009 – 06/30/2010 (Year 1)

\$ 116,283 07/01/2010 - 06/30/2011 (Year 2)

\$ 116,085 07/01/2011 - 06/30/2012 (Year 3)

Summary: Spinal Muscular Atrophy (SMA) patients have homozygous deletions or mutations in the survival motor neuron (SMN1) gene and retain at least one copy of the nearly identical SMN2 gene. The SMN2 gene produces reduced amounts of functional SMN that cannot fully compensate for the absence of SMN1, leading to specific degeneration of motor neurons in the spinal cord and atrophy of skeletal muscle.

Currently, most therapeutic strategies for SMA aim at increasing the expression of functional SMN from the SMN2 gene. The SMN protein has a well-established function in RNA metabolism that is regulated in a tissue-specific and time-dependent manner by unknown cellular factors. Our hypothesis is that targeting these factors will enhance the low levels of SMN activity in cells of SMA patients and represent an alternative therapeutic approach. The project will explore this possibility by studying the cellular factors that control SMN activity and their mechanism of action in motor neurons and skeletal muscle cells. The effect of modulating the activity of these regulatory factors in cellular and animal models of SMA will also be analyzed. Beyond providing fundamental insights into the biology of SMN, our studies will identify potential targets for the development of novel therapeutic approaches for SMA.

Eric Schon, Ph.D.

(RG) Pharmacological approaches to treat human mitochondrial diseases

\$	130,146	07/01/2009 – 06/30/2010	(Year 1)
\$	133,291	07/01/2010 - 06/30/2011	(Year 2)
\$	136,506	07/01/2011 - 06/30/2012	(Year 3)

Summary: Mitochondrial diseases can be due to mutations in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). We intend to develop pharmacological strategies to treat both types of disorder, using cellular and animal models. For mtDNA mutations (usually maternally-inherited), we propose to reduce the proportion of mutated mtDNAs below the threshold for dysfunction ("heteroplasmic shifting"), based on our previous findings and preliminary data suggesting that it may be possible to eliminate or decrease the number of mutant mtDNAs selectively, leaving the normal mtDNAs intact. Rapamycin, a drug that activates the cell's innate pathways for degrading unwanted materials (autophagy), exerts dramatic effects upon cells carrying mtDNA mutations. We will assess the ability of rapamycin and similar compounds to shift heteroplasmy in cells with known pathogenic mtDNA mutations. For nDNA mutations (mendelian-inherited; usually recessive), we will focus on a recent exciting finding that bezafibrate, a drug that stimulates mitochondrial proliferation, can rescue mitochondrial dysfunction in cellular and mouse models of respiratory chain deficiency. We will use bezafibrate (or related compounds) to assess whether we can rescue respiratory chain function in cells and mice with mutations in SCO2, a protein that, when mutated, causes mendelian-inherited encephalomyopathy due to dysfunction in complex IV (cytochrome c oxidase, or COX) of the respiratory chain.

Ji-Yeon Shin, Ph.D

(DG) LAP1 involvement in the pathology of Emery-Dreifuss muscular dystrophy

\$	60,000	07/01/2010 - 06/30/2011	(Year 1)
\$	60,000	07/01/2011 - 06/30/2012	(Year 2)
\$	60,000	07/01/2012 - 06/30/2013	(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.

Howard J. Worman, M.D.

(RG) Treatment of Cardiomyopathy in Emery-Dreifuss Muscular Dystrophy

\$ 103,631 07/01/2010 - 06/30/2011 (Year 1)

\$ 103,631 07/01/2011 - 06/30/2012 (Year 2)

\$ 103,631 07/01/2012 - 06/30/2013 (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 cardiomyopathy 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 - The Hospital for Special Surgery

Dale Lange, M.D., M.D.

(RG) Safety and Efficacy of SOD1 Inhibition by Pyrimethamine in Familial ALS

\$ 200,000 04/01/2009 – 03/31/2010 (Year 1)

\$ 190,000 04/01/2010 – 03/31/2011 (Year 2)

\$ 95,000 04/01/2011 – 03/31/2013 (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 - Joan & Sanford I. Weill Medical College of Cornell University

Giovanni Manfredi, M.D., Ph.D.

(RG) Amelioration of OXPHOS defects by mitochondrial soluble adenylyl cyclase

\$ 99,846 07/01/2009 – 06/30/2010 (Year 1)

\$ 99,846 07/01/2010 - 06/30/2011 (Year 2)

\$ 99,846 07/01/2011 - 06/30/2012 (Year 3)

Summary: Mitochondrial diseases, as a group, are among the most common hereditary disorders for which no effective treatment is currently available. They arise from mutations both in the nucleus and in the mitochondrial DNA (mtDNA), affecting genes encoding for the oxidative phosphorylation (OXPHOS) machinery. Mitochondria are the main cellular source of ATP, the molecule in which most of the cellular energy is stored. Thus, OXPHOS mutations result in bio-energetic defects that ultimately cause degeneration of many tissues, such as muscle, heart, and brain. Little is known on how mutant cells try to compensate for their metabolic defects. We know that phosphorylation of mitochondrial enzymes is one of the mechanism of regulation of metabolic activity. Cyclic AMP (cAMP) promotes protein phosphorylation and modulates enzymatic activity, but the source of cAMP in mitochondria is unknown. We have identified a novel cAMP-driven signaling pathway within mitochondria that works as a metabolic sensor. This pathway may participate to the compensatory responses to OXPHOS defects. We also demonstrated that OXPHOS defects could be ameliorated through this pathway. In this proposal, we will utilize the pathway to improve OXPHOS in mutant cells and in animal models of OXPHOS defects. Our goal is to identify new avenues for treatment of mitochondrial disorders.

New York - Memorial Sloan-Kettering Cancer Center

Sonja Nowotschin, Ph.D

(DG) Muscle morphogenesis: investigating cellular behaviors and molecular mechanisms

\$	54,546	08/01/2011 - 07/31/2012	(Year 1)
\$	54,546	08/01/2012 - 07/31/2013	(Year 2)
\$	54,546	08/01/2013 - 07/31/2014	(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.

Rochester - University of Rochester

Robert Griggs, M.D.

(RG) Recruitment for HYP HOP Phase III Trial in the Periodic Paralysis

\$	55,802	07/01/2008 – 06/30/2009	(Year 1)
\$	53,505	07/01/2009 – 06/30/2010	(Year 2)
\$	54,013	07/01/2010 – 06/30/2013	(Year 3)

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.

Masayuki Nakamori, M.D., Ph.D.

(DG) The role of repeat instability in development and senescence in DM1 cell

\$ 60,000 07/01/2009 – 06/30/2010 (Year 1)

\$ 60,000 07/01/2010 - 06/30/2011 (Year 2)

\$ 60,000 07/01/2011 - 06/30/2012 (Year 3)

Summary: The effects of myotonic dystrophy type 1 (DM1) on muscle are complex. Infants that are severely affected by DM1 have a problem with formation of muscle tissue (congenital DM1). The problem with muscle formation may also interfere with muscle healing in adults who have DM1. DM1 also causes a problem with the capacity of muscle cells for self-renewal. This problem is similar in some ways to premature aging. The goal of this project is to learn more about why myotonic dystrophy has these effects on muscle development and aging.

Araya Puwanant, M.D.

(CRTG) Disease Progression and Physiological Changes in Myotonic Dystrophy

\$ 90,000 07/01/2011 - 06/30/2012 (Year 1)

\$ 83,400 07/01/2012 - 06/30/2013 (Year 2)

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.

2012 ACTIVE GRANTS

OHIO

Cincinnati - University of Cincinnati

Rhett Kovall, Ph.D.

(RG) Analysis of Notch transcriptional mechanisms required for muscle regeneration

\$ 96,755 07/01/2009 – 06/30/2010 (Year 1)

\$ 93,939 07/01/2010 - 06/30/2011 (Year 2)

\$ 96,147 07/01/2011 - 06/30/2012 (Year 3)

Summary: Cells in our bodies that are destined to become muscle follow a distinct route of maturation from precursor cells that are controlled by cellular signaling pathways. Similar signaling mechanisms are employed to repair injured muscle, and are often defective in degenerative muscle conditions, such as muscular dystrophy. Therefore, understanding the cellular pathways that promote muscle maturation and repair hold great promise for developing new strategies for treating muscular dystrophies. The Notch signaling pathway is one such pathway that is critical for the maturation of precursor cells into muscle; however, our current understanding of Notch signaling and its role in muscle regeneration is inadequate for developing novel therapeutics to treat degenerative muscular conditions. The objective of our proposal is to bridge this gap in our understanding, providing biophysical data for Notch pathway transcription complexes, which will lay the groundwork for future drug discovery efforts.

Columbus - Research Institute at Nationwide Children's Hospital

Scott Harper, Ph.D.

(RG) Development of An Inducible FSHD Mouse Model

\$ 109,494 02/01/2012 - 01/31/2013 (Year 1)

\$ 99,222 02/01/2013 - 01/31/2014 (Year 2)

\$ 108,748 02/01/2014 - 01/31/2015 (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.

Paul Martin, Ph.D.

(RG) Protein-based GALGT2 therapies for Duchenne muscular dystrophy

\$ 132,000 08/01/2011 - 07/31/2012 (Year 1)

\$ 132,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 132,000 08/01/2013 - 07/31/2014 (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., M.D.

(RG) Supplemental Support for AVI-4658 Phase II Clinical Trial

\$ 110,000 08/01/2011 - 07/31/2012 (Year 1)

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.

Jerry Mendell, M.D., M.D.

(RG) Treatment of the Dystrophin Deficient Cardiomyopathy

\$ 315,176 07/01/2009 - 06/30/2010 (Year 1)

\$ 306,805 07/01/2010 - 06/30/2011 (Year 2)

\$ 309,841 07/01/2011 - 06/30/2012 (Year 3)

Summary: The heart of Duchenne muscular dystrophy (DMD) patients requires more study. Although many promising treatments are evolving for skeletal muscle, the therapeutic approach for cardiac muscle with dystrophin deficiency remains limited. In a newly established 5 Center MDA Network we hope to enhance treatments for cardiac muscle through the following efforts: 1) establish standards for Doppler-echocardiography to facilitate reliability of testing between participating centers; 2) compare the efficacy of the angiotensin converting enzyme inhibitor lisinopril with angiotensin II receptor antagonist losartan for the cardiomyopathy of DMD; 3) correlate the severity of the cardiomyopathy with dystrophin gene mutations and skeletal muscle function. The proposed studies will establish the standard of care and best treatment for the cardiomyopathy of DMD.

Christopher Pierson, M.D., Ph.D.

(RG) Follistatin Gene Therapy in Mouse Models of Myotubular Myopathy

\$ 85,613 01/01/2010 - 12/31/2010 (Year 1)

\$ 85,613 01/01/2011 - 12/31/2011 (Year 2)

\$ 85,613 01/01/2012 - 12/31/2012 (Year 3)

Summary: Myotubular myopathy causes severe muscle weakness over the entire body. Due to breathing problems, many affected patients need to be on a ventilator and die early in life. Affected patients have very small muscles, and it has been suggested that treatments aiming to make muscles bigger should be explored; however, this has to be done safely. For example, using steroids to increase muscle size is an option, but they are dangerous, especially with long-term use. In order to treat myotubular myopathy, we would need something that would have a persistent effect on muscle growth and would be safe for an extended period of time. We aim to develop a new therapeutic treatment that involves inhibiting myostatin activity. Myostatin is a protein that limits muscle size, and its activity can be inhibited by another protein called follistatin. We plan on using gene therapy to give follistatin to mice with myotubular myopathy, and expect to make their muscles bigger and hopefully extend their life span. A previous study safely used gene therapy to increase follistatin levels and enhance muscle size and strength in mice with muscular dystrophy. We plan to use this same approach in both of our mouse models of myotubular myopathy to increase muscle mass, strength and hopefully, extend the lifespan of the mice.

Columbus - The Ohio State University (OSU)

Arthur Burghes, Ph.D.

(RG) How do SMN missense mutations affect RNP assembly and how does this cause SMA?
 \$ 107,321 07/01/2009 - 06/30/2010 (Year 1)
 \$ 104,461 07/01/2010 - 06/30/2011 (Year 2)
 \$ 103,892 07/01/2011 - 06/30/2012 (Year 3)

Summary: Spinal muscular atrophy is caused by loss or mutation of the SMN1 gene and retention of the SMN2 gene. This results in insufficient SMN levels for motor neurons and spinal muscular atrophy. Loss of SMN, a ubiquitously expressed protein, is lethal. Therefore SMA is caused by a reduction of SMN levels and not by the complete loss of SMN. We have created mice with low levels of SMN thus recapitulating the situation that occurs in human SMA patients, and resulting in a mouse model of SMA. These SMA mice lack mouse *Smn* and contain human SMN2. Although often misrepresented, SMN2 produces sufficient SMN levels for the normal function of most tissues. However, SMN2 does not produce sufficient SMN for specific neuronal populations such as the motor neurons. This is most vividly demonstrated by the correction of SMA mice with high expression of SMN in the neural system but not other tissues. Thus, low SMN levels in most tissues other than neuron have a limited consequence. SMN is known to be important for assembly of molecules critical for gene expression. No study to date has performed a reliable and rigorous analysis of gene expression changes with adequate controls. We will use specific SMN mutations to define the function disrupted in SMA. We will define the expression changes that are specific to SMA and how they relate to severity. This will define how reduced SMN gives rise to SMA.

Jill Rafael-Fortney, Ph.D.

(RG) Investigation of a new treatment target for heart failure in muscular dystrophy
 \$ 126,923 01/01/2010 - 12/31/2010 (Year 1)
 \$ 126,923 01/01/2011 - 12/31/2011 (Year 2)
 \$ 126,923 01/01/2012 - 12/31/2012 (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.

Dayton - Wright State University

Mark Rich, M.D., Ph.D.

(RG) Treating myotonia congenita
 \$ 53,358 02/01/2011 - 01/31/2012 (Year 1)

Summary: Myotonia congenita is an inherited muscle disease in which muscle is stiff. The cause of stiffness is a mutation in a protein that is involved in electrical signaling in muscle. A clinical feature of the disease is that when patients exercise their stiffness gets better. Unfortunately this effect is temporary and reverses as soon as patients rest. There is a mouse model of myotonia congenita that also has muscle stiffness. We have been working on this mouse model and believe we are close to figuring out why exercise lessens muscle stiffness. Our goal is to determine whether our idea is correct. If so there are drugs that can be used to treat muscle stiffness in patients with myotonia congenita. The drugs are already used in people to treat other disorders. We will try these drugs in the mice to see if they reduces their stiffness. The long-term goal is to try the drugs in patients.

Toledo - The University of Toledo

Kenneth Hensley, Ph.D.

(RG) Targeting CRMP2 to Treat Motor Neuron Disease
 \$ 110,679 02/01/2012 - 01/31/2013 (Year 1)
 \$ 108,868 02/01/2013 - 01/31/2014 (Year 2)
 \$ 108,606 02/01/2014 - 01/31/2015 (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.

2012 ACTIVE GRANTS

PENNSYLVANIA

Doylestown - ALS Biopharma, LLC

Allen Reitz,

(MVP) Development of heat shock protein 70-based therapy for amyotrophic lateral sclerosis

\$ 170,000 03/01/2011 – 12/31/2011 (Year 1)

\$ 80,000 01/01/2012 - 12/31/2012 (Year 2)

Summary: Page 1 of 1 Development of a heat shock protein 70-based therapy for amyotrophic lateral sclerosis. Lay Abstract. We seek to provide rapid therapeutic relief to patients suffering from the debilitating and fatal condition of amyotrophic lateral sclerosis (ALS). Degeneration of motoneurons causes weakness in the limbs and extremities of ALS patients that leads to respiratory failure and death, typically within 3–5 years of diagnosis. We have demonstrated that exogenously administered recombinant human heat shock protein 70 (Hsp70; 20 µg i.p., 3X weekly) shows strong efficacy in the SOD1G93A mouse, the most commonly used animal model of ALS. Hsp70 is 11X and 9X more effective in delaying time of onset of symptoms and mortality in this model, respectively, than riluzole, the only standard of care for ALS. Hsp70 is a stress response protein that functions inside and outside the cell to clear misfolded proteins and block cell death. Motoneurons are particularly vulnerable in ALS

Philadelphia - The Children's Hospital of Philadelphia

David Lynch, MD, PhD

(RG) Insulin resistance in Friedreich ataxia

\$ 101,111 08/01/2011 - 07/31/2012 (Year 1)

\$ 101,111 08/01/2012 - 07/31/2013 (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 - Philadelphia Health and Education Corporation d/b/a Drexel University College of Medicine

Terry Heiman-Patterson, M.D., MD

(RG) Identification of modifying genes in murine models of ALS

\$ 110,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 110,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 110,000 02/01/2014 - 01/31/2015 (Year 3)

Summary: A mouse model for ALS has been created that carries the human form of an ALS gene (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 02/01/2011 - 01/31/2012 (Year 1)
 \$ 125,000 02/01/2012 - 01/31/2013 (Year 2)
 \$ 125,000 02/01/2013 - 01/31/2014 (Year 3)

Summary: 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 - University of Pennsylvania

Nancy M. Bonini, Ph.D.

(RG) Novel pathway and compound modifiers for myotonic dystrophy
 \$ 87,020 07/01/2009 - 06/30/2010 (Year 1)
 \$ 75,534 07/01/2010 - 06/30/2011 (Year 2)
 \$ 77,774 07/01/2011 - 06/30/2012 (Year 3)

Summary: My laboratory has developed a model for myotonic dystrophy in a powerful model organism, the fly *Drosophila*. The myotonic dystrophy model my laboratory developed recapitulates key aspects of human myotonic dystrophy in remarkable detail. We have used this model to define new pathways that contribute to myotonic dystrophy. We now propose to define specifically the nature of those pathways, define new pathways, and use this model for compound and drug screening. These discoveries will provide the foundation for new treatments for myotonic dystrophy.

Michael Granato, Ph.D.

- (RG) Zebrafish models of motor neuron and peripheral nerve diseases
- | | | | |
|----|---------|-------------------------|----------|
| \$ | 133,391 | 07/01/2009 - 06/30/2010 | (Year 1) |
| \$ | 99,827 | 07/01/2010 - 06/30/2011 | (Year 2) |
| \$ | 104,082 | 07/01/2011 - 06/30/2012 | (Year 3) |

Summary: Control of body musculature requires the interplay between the nervous system and the body musculature. Motor neurons form the 'interface' as their axons connect the nervous system with the muscle. Breakdown, degeneration and loss of motor axons is a hallmark of many hereditary neuropathies including Charcot-Marie-Tooth (CMT) disease, and motoneuron diseases such as Amyotrophic Lateral Sclerosis. Thus, axonal loss is a key determinant of clinical disability in hereditary neuropathies, and prevention of axonal loss is one of the most important therapeutic strategies currently being considered. To develop successful therapeutic strategies, it is therefore essential to focus on the events at the onset of the disease, including gradual axonal loss or degeneration. Because it is essential but technically very difficult to visualize this process inside animals, the goal of this proposal is to use the transparent zebrafish as a model in which to visualize and genetically characterize the axonal degeneration process. The optical transparency of the larvae and the relatively simple nervous system make this an outstanding model organism. Understanding the process of axonal degeneration will provide a powerful platform to directly test new therapeutic compounds to attenuate axonal degeneration and motor neuron loss.

Tejvir Khurana, M.D. , Ph.D.

- (RG) Utrophin upregulation via microRNA repression as a therapy for DMD
- | | | | |
|----|---------|-------------------------|----------|
| \$ | 126,500 | 08/01/2011 - 07/31/2012 | (Year 1) |
| \$ | 126,500 | 08/01/2012 - 07/31/2013 | (Year 2) |
| \$ | 126,500 | 08/01/2013 - 07/31/2014 | (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.

Todd Lamitina, Ph.D.

- (RG) Evolutionarily conserved synaptic function of Dysferlin in *C. elegans* and mice
- | | | | |
|----|---------|-------------------------|----------|
| \$ | 100,000 | 07/01/2009 - 06/30/2010 | (Year 1) |
| \$ | 100,000 | 07/01/2010 - 06/30/2011 | (Year 2) |
| \$ | 100,000 | 07/01/2011 - 06/30/2012 | (Year 3) |

Summary: How and why mutations in dysferlin cause Limb-Girdle Muscular Dystrophy type 2B, or Dysferlinopathy, is poorly understood. The small model worm *C. elegans* expresses a Dysferlin gene that is highly similar to human Dysferlin and has nerves and muscle that are remarkably similar to their human counterparts. We have discovered that mutations in *C. elegans* Dysferlin cause defects in the communication between neurons and muscle. We have also discovered that similar defects are present in the muscle of Dysferlin mutant mice, suggesting that the function(s) of Dysferlin are evolutionarily conserved between *C. elegans* and mammals. Interestingly, similar defects underlie other muscle disorders, but a role for Dysferlin in muscle-neuron communication has never been proposed. Such defects are amenable to therapies that

might prove beneficial to LGDM2B patients. In this proposal, we will 1) define the molecular role of Dysferlin in *C. elegans*, 2) test mechanisms for how Dysferlin may regulated muscle-neuron communication in worms and mice and 3) thoroughly characterize the synaptic defects caused by Dysferlin mutations in mammalian muscle. Our studies have the potential to define a new mechanism by which mutations in Dysferlin cause LGMD2B and may help to define a new treatment strategy for this disease.

Jon Lindstrom, Ph.D.

(RG) Specific Immunosuppression of EAMG

\$	150,000	02/01/2011 - 01/31/2012	(Year 1)
\$	150,000	02/01/2012 - 01/31/2013	(Year 2)
\$	150,000	02/01/2013 - 01/31/2014	(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.

Philadelphia - Thomas Jefferson University

Ya-Ming Hou, Ph.D.

(RG) Roles of aminoacyl-tRNA synthetases in peripheral neuropathy

\$	108,720	01/01/2010 - 12/31/2010	(Year 1)
\$	108,720	01/01/2011 - 12/31/2011	(Year 2)
\$	108,720	01/01/2012 - 12/31/2012	(Year 3)

Summary: This project will investigate the roles of aminoacyl-tRNA synthetases (aaRSs) in the development of the Charcot-Marie-Tooth (CMT) disease. Some CMT mutations are identified in aaRSs, which are key enzymes in decoding of genetic information. The connection between aaRSs and CMT disease suggests the possibility that the decoding machinery is linked to neuron health. One aim of this project is to test if CMT mutations impair the activity and quality of aaRSs, while another is to test if CMT mutations cause aberrant structures of aaRSs. Together, these aims will provide a strong foundation for developing new therapies for the CMT disease.

Dena Jacob, Ph.D.

(DG) Multi-drug resistance in amyotrophic lateral sclerosis: implications for therapy

\$	60,000	07/01/2010 - 06/30/2011	(Year 1)
\$	60,000	07/01/2011 - 06/30/2012	(Year 2)
\$	60,000	07/01/2012 - 06/30/2013	(Year 3)

Summary: Despite numerous drug trials to cure the mouse that models amyotrophic lateral 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.

2012 ACTIVE GRANTS

TENNESSEE

Memphis - St. Jude Children's Research Hospital

Brian Freibaum, Ph.D

(DG)	Characterizing the role of TDP-43 in ALS		
\$	60,000	07/01/2010 - 06/30/2011	(Year 1)
\$	60,000	07/01/2011 - 06/30/2012	(Year 2)
\$	60,000	07/01/2012 - 06/30/2013	(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.

J. Taylor, MD, PhD

(RG)	The molecular pathogenesis of spinobulbar muscular atrophy		
\$	110,000	02/01/2011 - 01/31/2012	(Year 1)
\$	110,000	02/01/2012 - 01/31/2013	(Year 2)
\$	110,000	02/01/2013 - 01/31/2014	(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

2012 ACTIVE GRANTS

TEXAS

Dallas - UT Southwestern Medical Center

Steve Cannon, MD, PhD

(RG) Disease pathogenesis and modification in a mouse model of HypoPP

\$ 138,776 08/01/2011 - 07/31/2012 (Year 1)

\$ 132,749 08/01/2012 - 07/31/2013 (Year 2)

\$ 132,749 08/01/2013 - 07/31/2014 (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.

Steve Cannon, MD, PhD

(RG) Mechanistic Studies on the Pathophysiology of Hypokalemic Periodic Paralysis

\$ 52,180 07/01/2009 - 06/30/2010 (Year 1)

\$ 26,428 07/01/2010 - 06/30/2011 (Year 2)

\$ 26,428 07/01/2011 - 06/30/2012 (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 transient loss of muscle electrical excitability. In addition, some patients develop a late-onset permanent weakness in the legs that may prevent independent ambulation. The gene defect underlying the most commonly occurring form of periodic paralysis was identified 15 years ago, and yet we still do not understand how mutations in this calcium channel lead to a transient loss of muscle excitability. To gain further insight on the mechanistic basis for periodic paralysis, we have developed an animal model by generating mice that have the analogous mutation of the skeletal muscle calcium channel gene that causes the most common form of human hypokalemic periodic paralysis. The studies in this proposal will characterize the extent to which the mouse model emulates the human disease, will define the link between altered function of mutant calcium channels and disruption of muscle excitability, and will enable us to study the mechanism by which drugs found empirically to be of partial benefit act and thereby promote a more systematic approach toward improving therapy in these debilitating disorders.

Jeffrey Elliott, M.D., M.D.

(RRG) Animal models in ALS

\$ 15,293 04/01/2011 – 03/31/2012 (Year 1)

Summary: Abnormalities in the processing of TDP-43 may be critical in the pathogenesis of both familial and sporadic ALS. We have recently published an initial description of several transgenic mouse lines expressing human wild type or A315T (non-fused or native) TDP-43. These mice develop progressive motor phenotypes characterized by cytoplasmic ubiquitin inclusions, motor neuron loss and extensive gliosis in a pathology that closely mimics the human condition. In addition, these mice also reproduce the extra-nuclear mis-localization and fragmentation observed in human tissue providing further evidence that these transgenic mice can serve as excellent models of human ALS. In this proposal we wish to extend our characterization and description of these mouse lines by undertaking detailed immuno-fluorescent studies of fixed spinal cord sections and western blots of frozen spinal cord extracts with antibodies directed against key proteins that may be linked to TDP-43 function. This data may provide further insights into pathways mediating TDP-43 induced motor neuron disease.

Jeffrey Elliott, M.D., M.D.

(RRG) The isolation of TDP-43 fragments

\$ 121,836 09/01/2011 – 08/31/2012 (Year 1)

Summary: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurological disease of motor nerve cells. 5% of ALS is hereditary, while the vast majority occurs sporadically in previously healthy individuals. Recent pathological evidence from human autopsy studies has suggested that alterations in the protein TDP-43 may be critical in causing motor nerve cell dysfunction in ALS. In particular, the cleavage of full length TDP-43 into smaller sized fragments, which accumulate within the nerve cell cytoplasm, appear to correlate strongly with disease progression. Currently, the precise nature of these smaller TDP-43 fragments and what generates them is unknown. To further understand the biological role of TDP-43 in disease, we propose to purify and isolate these TDP-43 fragments which will allow for their eventual sequencing. Identifying the sequences of the various TD-43 fragments will allow for great insights into the abnormal processing of TDP-43 in ALS and for potential therapeutic targets.

Ronald Haller, M.D.

(RG) Impaired Oxidative Capacity in McArdle Disease: Causes and Treatment

\$ 127,700 01/01/2010 - 12/31/2010 (Year 1)

\$ 127,700 01/01/2011 - 12/31/2011 (Year 2)

\$ 127,700 01/01/2012 - 12/31/2012 (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.

Galveston - The University of Texas Medical Branch

Premkumar Christadoss, M.B.B.S

(RG) Complement siRNA gene therapy for myasthenia gravis

\$ 130,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 130,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 130,000 02/01/2014 - 01/31/2015 (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.

Premkumar Christadoss, M.B.B.S

(RG) Innate Immunity in Autoimmune Myasthenia Gravis Pathogenesis

\$ 125,000 01/01/2009 - 12/31/2009 (Year 1)

\$ 118,750 01/01/2010 - 12/31/2010 (Year 2)

\$ 118,750 01/01/2011 - 01/31/2012 (Year 3)

Summary: Myasthenia Gravis (MG) is often preceded by infection. We hypothesize that chemicals and/or proteins expressed in infectious agents act as adjuvants in triggering autoimmune (self reactive) response, culminating in MG. We have developed a new mouse model of MG by immunizing mice with a chemical derived from E.coli called lipopolysaccharide (LPS) with acetylcholine receptor protein. In this model antibodies to acetylcholine receptors are produced without CD4 cell help. We will study the immunological mechanisms by which LPS triggers autoimmune MG. These studies would lead to specific therapy of MG by inhibiting the pathway of pathogenic antibody production in myasthenia gravis.

Houston - Baylor College of Medicine

Thomas Cooper, M.D.

(RG) Oligonucleotide-based therapy for myotonic dystrophy

\$ 115,354 01/01/2010 - 12/31/2010 (Year 1)

\$ 112,794 01/01/2011 - 12/31/2011 (Year 2)

\$ 112,794 01/01/2012 - 12/31/2012 (Year 3)

Summary: There are two types of myotonic dystrophy and both are caused by an unusual type of mutation in which a small segment of the mutated gene is repeated multiple times. In myotonic dystrophy, these repeated segments cause the RNA from the mutated gene to get stuck in the nucleus. This RNA is toxic and disrupts the normal processes required for expression of many genes. The myotonic dystrophy mutation is particularly harmful because it alters the normal function of many genes, not just the gene with the mutation. The goal of this project is to develop short DNA oligonucleotides (oligos) that will degrade the toxic RNA containing the repeated segment. Our plan is to first test different chemical structures for the DNA using the cell culture model system and determine which are best at degrading the RNA. We will then test the DNA oligos that worked best in mouse models that we developed (with previous support from the MDA) in which the toxic RNA is expressed in heart or skeletal muscle. This approach will be developed for the more common form of myotonic dystrophy (type 1) but will also be applicable to type 2. The primary goal of this project is to develop these mouse models to test different therapeutic approaches.

Maria de Haro, Ph.D

(DG) Novel genes suppress CUG-induced muscle wasting

\$	60,000	01/01/2010 - 12/31/2010	(Year 1)
\$	60,000	01/01/2011 - 12/31/2011	(Year 2)
\$	60,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: Myotonic Dystrophy Type 1 (DM1) is caused by a CTG expansion in the 3' UTR region of the DMPK gene. This mutation causes a gain-of-function of the expanded RNA containing CUG repeats, which sequesters RNA-binding proteins like Muscleblind, and causes alterations in the levels of other proteins such as CUG-BP1. My preliminary work led to the publication of the first model of DM1 in *Drosophila*, which recapitulates key features of the disease such as muscle wasting and accumulation of the expanded CUG-containing transcript in nuclear foci. Furthermore, we were able to modify these phenotypes by altering the levels of Muscleblind and CUG-BP1, two key players in DM1 pathogenesis. Since the publication of the *Drosophila* DM1 model, I have developed additional behavioral and molecular assays. I have identified changes in the alternative splicing of specific muscle genes recapitulating what is observed in DM1 patients. I also carried out a genetic screen to identify new modifier genes using a collection of mutations in RNA-binding proteins in *Drosophila*. This screen identified five genes that modify the muscle phenotype. This investigation will further characterize and validate these novel modifier genes using behavioral and molecular assays to investigate novel genetic pathways involved in DM1 pathogenesis. I will also test the modifier genes in a mammalian model of DM1.

Susan Hamilton, Ph.D.

(RG) Central Core Disease and Mitochondrial Dysfunction

\$	125,000	08/01/2011 - 07/31/2012	(Year 1)
\$	125,000	08/01/2012 - 07/31/2013	(Year 2)
\$	125,000	08/01/2013 - 07/31/2014	(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 Wehrens, M.D., Ph.D.

(RG) Mechanisms underlying arrhythmias and heart failure in Muscular Dystrophy

\$	104,500	02/01/2011 - 01/31/2012	(Year 1)
\$	104,500	02/01/2012 - 01/31/2013	(Year 2)
\$	104,500	02/01/2013 - 01/31/2014	(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 studied 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 Wong, Ph.D.

(RG) Innovative one-step diagnosis of complex mitochondrial disorders

\$ 137,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 137,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 137,000 07/01/2012 - 06/30/2013 (Year 3)

Summary: Mitochondria are the cellular organelles where energy currency, ATP, is produced. 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 - Methodist Neurological Institute

Stanley Appel, M.D., MD

(RG) Immune Mechanisms in Amyotrophic Lateral Sclerosis

\$ 110,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 110,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 110,000 07/01/2012 - 06/30/2013 (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

Raymond Grill, PhD

(RG) Targeting inflammation in ALS using a dual COX/LOX inhibitor
\$ 106,426 08/01/2011 - 07/31/2012 (Year 1)
\$ 96,082 08/01/2012 - 07/31/2013 (Year 2)

Summary: Riluzole is the sole FDA-approved treatment for ALS. However, Riluzole treatment 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 blood-brain-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 08/01/2011 - 07/31/2012 (Year 1)
\$ 98,061 08/01/2012 - 07/31/2013 (Year 2)
\$ 98,061 08/01/2013 - 07/31/2014 (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 alpha-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors has been observed in patients with sporadic ALS, implicating these receptors in the pathological process of neurodegeneration. 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 Narkar, Ph.D

(RG) Regulation of oxidative slow-twitch muscles by ERRgamma in DMD

\$ 111,822 07/01/2010 - 06/30/2011 (Year 1)

\$ 95,252 07/01/2011 - 06/30/2012 (Year 2)

\$ 95,252 07/01/2012 - 06/30/2013 (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.

Irina Serysheva, Ph.D.

(RG) Structural studies of skeletal muscle RyR channel

\$ 115,988 07/01/2009 - 06/30/2010 (Year 1)

\$ 117,802 07/01/2010 - 06/30/2011 (Year 2)

\$ 118,945 07/01/2011 - 06/30/2012 (Year 3)

Summary: Numerous physiological processes are controlled by Ca²⁺, including muscle contraction and heartbeat. Intracellular Ca²⁺ levels are regulated by 'Ca channels', specialized proteins that allow Ca²⁺ to pass through cell membranes. Dysfunction of these channels leads to a wide array of diseases, such as muscle and cardiovascular disorders. Many drugs currently in use to treat these diseases target Ca²⁺ channels. Due to high prevalence of muscle disorders in the USA and worldwide, new ways to interfere with Ca²⁺ channels in disease states are needed. Design of such strategies, however, is hampered by the lack of sufficient knowledge about the architecture of these proteins. In this particular project, we study the type 1 ryanodine receptor (RyR1), the main Ca²⁺ release channel in skeletal muscle. RyR1 mediates Ca²⁺ release from the sarcoplasmic reticulum to initiate muscle contraction. Our major approach consists of electron cryomicroscopy, a technique that allows us to elucidate the structure of a protein at subnanometer resolution. The resulting structures are excellent templates for the design of novel drugs, and provide invaluable information about how RyR1 works. We also assess RyR1 function using radioligand binding assays and measuring Ca²⁺ fluxes through channels reconstituted into lipid vesicles. By combining these techniques, we aim to increase our understanding of Ca²⁺ release channels, laying the basis for novel strategies to interfere with their function in disease.

Eric Wagner, Ph.D.

(RG) Developing Dux4 3' End Formation Molecular Antagonists

\$	96,160	08/01/2011 - 07/31/2012	(Year 1)
\$	93,542	08/01/2012 - 07/31/2013	(Year 2)
\$	93,542	08/01/2013 - 07/31/2014	(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.

2012 ACTIVE GRANTS

UTAH

Salt Lake City - Sfida BioLogic, Inc.

John Manfredi, Ph.D.

(RG) Evaluation in Zebrafish of Small Molecules as SMA Therapeutics

\$ 63,257 07/01/2010 - 06/30/2011 (Year 1)

\$ 16,020 07/01/2011 - 06/30/2012 (Year 2)

Summary: We have identified novel compounds that promote the growth and function of motor neurons, including motor neurons of the spinal cord. The effects of the compounds on spinal motor neurons admit the possibility that the compounds could benefit those who suffer spinal muscular atrophy (SMA), a disorder distinguished by degeneration of spinal motor neurons. Furthermore, the compounds exhibit drug-like properties that suggest that they can be administered orally, for example as a pill. Finally, the compounds work by a mechanism that is unique among experimental treatments of SMA. Collectively, these neurological, pharmaceutical, and mechanistic features of the compounds argue for evaluating their potential as therapeutics for SMA. We will evaluate their therapeutic potential using zebrafish that have been genetically engineered to simulate aspects of SMA. By testing the compounds in this economical model we will determine if the neurologic activities of the compounds are relevant to SMA. If so, the results will justify future tests of the compounds using more authentic and expensive models of SMA.

Salt Lake City - University of Utah

Gabrielle Kardon, Ph.D.

(RG) Role of Connective Tissue Fibroblasts in Muscle Regeneration

\$ 118,569 07/01/2009 - 06/30/2010 (Year 1)

\$ 148,942 07/01/2010 - 06/30/2011 (Year 2)

\$ 146,418 07/01/2011 - 06/30/2012 (Year 3)

Summary: Duchenne Muscular Dystrophy (DMD) is characterized by pathological muscle degeneration and regeneration and connective tissue fibrosis. Muscle regeneration is mediated by resident stem cells called satellite cells. In DMD repeated rounds of regeneration lead to the diminished proliferation and regenerative capacity of the satellite cells and ultimately to the loss of muscle mass and function. Concurrent with the loss of muscle mass there is an excessive increase in muscle connective tissue, termed fibrosis, which further impairs muscle function. We propose that the fibroblasts embedded in the connective tissue contribute significantly to the pathology of DMD. These fibroblasts may regulate satellite cells and are likely to be the source of connective tissue fibrosis. Study of these connective tissue fibroblasts has been severely hampered by the lack of molecular markers for these cells. Recently we have identified the gene Tcf4 as a robust marker of these fibroblasts. We propose to use mouse genetics to test the role of Tcf4+ connective tissue fibroblasts in regulating satellite cells and fibrosis during muscle regeneration. In addition, we will test the role of an important signaling molecule, β -catenin, in satellite cells, fibroblasts, and muscle cells in

muscle regeneration. Results from our research will give us insights into the cell-cell and cell-connective tissue interactions governing normal muscle regeneration and the pathology of DMD.

Kathryn Swoboda, M.D.

(RG) Phase I/II Study of NaPB in Pre-symptomatic Infants with SMA: STOP SMA STUDY

\$ 111,144 01/01/2009 - 12/31/2009 (Year 1)

\$ 118,159 01/01/2010 - 12/31/2010 (Year 2)

\$ 103,312 01/01/2011 - 12/31/2012 (Year 3)

Summary: Most babies with SMA are normal or nearly normal as newborns. However, babies who are at risk to develop SMA type I or type II typically develop significant weakness in the first few weeks or months of life. STOP SMA is a clinical trial designed to assess safety and effectiveness of sodium phenylbutyrate (NAPB) in infants confirmed to have SMA by genetic testing. Eligible infants are those likely to develop either type I or type II SMA because they have an affected older sibling and have tested positive for SMA via genetic testing. Babies predicted to develop SMA type I must be enrolled by 3 months of age, and babies predicted to develop SMA type II must be enrolled by 6 months of age. All babies will receive study drug, and we will assess safety and possible effectiveness by comparing them to children in our natural history database.

2012 ACTIVE GRANTS

VIRGINIA

Charlottesville - University of Virginia

Mani Mahadevan, M.D.

(RG) Characterizing RNA Foci in DM1

\$ 145,000 02/01/2011 - 01/31/2012 (Year 1)

\$ 145,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 145,000 02/01/2013 - 01/31/2014 (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 Mahadevan, M.D.

(RG) Identification of small molecule modulators of myogenic defects in DM1

\$ 140,676 02/01/2012 - 01/31/2013 (Year 1)

\$ 140,676 02/01/2013 - 01/31/2014 (Year 2)

Summary: The aim of this proposal is to identify potential therapies for myotonic dystrophy type 1 (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.

2012 ACTIVE GRANTS

WASHINGTON

Pullman - Washington State University

Buel Rodgers, Ph.D.

(RIG) Washington Center for Muscle Biology, Exercise Physiology Phenotyping Core

\$ 130,346 11/01/2011 – 10/31/2012 (Year 1)

\$ 71,171 11/01/2012 – 10/31/2013 (Year 2)

\$ 73,965 11/01/2013 – 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 - Seattle Institute for Biomedical and Clinical Research

Brian Kraemer, Ph.D.

(RG) Modulating TDP-43 phosphorylation for motor neuron protection in ALS

\$ 105,519 02/01/2012 - 01/31/2013 (Year 1)

\$ 105,519 02/01/2013 - 01/31/2014 (Year 2)

\$ 105,519 02/01/2014 - 01/31/2015 (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

Glen Banks, Ph.D.

(DG)	Skeletal muscle replacement in mdx mice with muscle stem cells		
\$	60,000	01/01/2010 - 12/31/2010	(Year 1)
\$	60,000	01/01/2011 - 12/31/2011	(Year 2)
\$	60,000	01/01/2012 - 12/31/2012	(Year 3)

Summary: Skeletal muscles contain stem cells that have the ability to regenerate large regions of muscle after injury or during disease. These stem cells can be taken from a mouse model of Duchenne muscular dystrophy, made to express dystrophin by genetic engineering and placed back into the same mouse to repopulate the dystrophic muscle with functioning muscles. The genetically corrected stem cells primarily fix the dystrophic muscles undergoing regeneration. Unfortunately, the regenerative potential of skeletal muscles diminishes with age in DMD, and most of the muscles are replaced by fibrotic tissue and fat tissue. Consequently DMD patients with advanced stages of disease are less amenable to stem cell therapy, unless the stem cells can form muscle fibers on their own. We and others have shown that at least some of these stem cells can form muscles fibers on their own, however it has not been established whether these cells can survive for long periods of time and contribute to muscle contraction. Our goals for this study are to examine whether these muscle fibers can contribute to contraction by forming connections with the motor neurons and the tendons while resisting injury. If the transplanted muscles can contribute to muscle contraction without joining to pre-existing muscle fibers, then it is possible that muscle disease can be partially reversed.

Jeffrey Chamberlain, Ph.D.

(RRG)	Systemic delivery of AAV vectors to muscle		
\$	201,136	02/01/2011 - 01/31/2012	(Year 1)

Summary: Duchenne muscular dystrophy (DMD) is a devastating x-linked recessive genetic disease. Affected children present with progressive muscle wasting, typically use wheelchairs by age 12, and often require respirators and cardiac support by the late teens to late twenties. There is no cure, and current therapies are only able to slow disease progression and provide postural and breathing support. Gene therapy would be an ideal solution for this genetic disease. Finding a way to deliver the genetic therapy to every muscle cell in the body, having the gene work, 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 essentially cure DMD in mice by whole body delivery of a new dystrophin gene carried in a delivery shuttle vector known as AAV. This application is to follow-up preliminary data showing that the method might be scaled-up, and to determine the feasibility of bring this treatment method into the clinic.

Joel Chamberlain, Ph.D.

(RG) RNA interference as an investigative and therapeutic tool for FSHD, FSHD RFA

\$ 100,000 04/01/2010 – 03/31/2011 (Year 1)

\$ 100,000 04/01/2011 – 03/31/2012 (Year 2)

Summary: The disease mechanism central to the cause of FSHD is not completely understood, but recent advances indicate that DNA sequence changes causes cells to produce protein that is toxic to muscle cell survival. Our goal is to develop a technology that could both give insight regarding the cause of disease as well as a potential route to therapy. The technology we are developing is related to a fundamental pathway that all cells use to function referred to as RNA interference (RNAi). RNAi is a process whereby cells fine-tune their levels of proteins that carry out the workload of maintaining body functions. We are developing ways to harness the potential for directed RNAi to turn off production of specific proteins in muscle that are thought to cause FSHD. Also, in targeting production of disease candidate proteins, we expect to learn more about what makes FSHD stem cells in culture appear different from normal cells. We also intend to examine specific DNA regions that could influence events that lead to FSHD. Once a therapeutic target is defined a significant challenge will be delivery of the therapy to muscle. Toward this goal we are continuing development of a therapeutic delivery system for muscles affected by FSHD using a shuttle virus that can infect muscles efficiently. We are positioned to better define the mechanisms of disease, to develop a means for treating the disease, and to deliver our therapy to the muscles weakened by FSHD.

Stephen Hauschka, Ph.D.

(RG) Regulatory Cassettes for Treating Diseased Cardiac Muscle

\$ 102,260 07/01/2009 - 06/30/2010 (Year 1)

\$ 105,311 07/01/2010 - 06/30/2011 (Year 2)

\$ 108,452 07/01/2011 - 06/30/2012 (Year 3)

Summary: This project will design and test molecular switches, so-called regulatory gene cassettes, that can be used to treat the cardiac muscles of patients with many neuromuscular diseases; e.g., DMD, Myotonic dystrophy, Pompe disease, and others. Cardiac muscle is often among the most severely affected muscles in neuromuscular disease, and its compromised function impacts the quality of life and longevity. Special versions of regulatory cassettes are designed for treating cardiac muscle because the transcription factor proteins that activate the molecular switches of cardiac genes differ from those in the arm and leg muscles. To create functional therapeutic genes, the molecular switches are connected to gene sequences that encode the proteins that are deficient or missing in neuromuscular diseases, e.g., dystrophin in DMD. When such therapeutic genes are inserted into muscle cells, or stem cells that develop into muscle, the switches turn on. This causes the normal protein to be made, and if produced in sufficient quantities this hopefully ameliorates the patient's cardiac problems. Regulatory cassettes will initially be designed and tested in neonatal rat cardiomyocytes; the best molecular switches will then be tested for high expression and therapeutic benefits in mouse muscle disease models. The best of these cassettes will then be re-designed as needed to obtain optimal expression levels in human cardiomyocytes.

Leo Pallanck, Ph.D.

(RG) Influence of the Mitochondrial Quality Control System on Mitochondrial Myopathy

\$ 104,233 07/01/2010 - 06/30/2011 (Year 1)

\$ 104,233 07/01/2011 - 06/30/2012 (Year 2)

\$ 104,233 07/01/2012 - 06/30/2013 (Year 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.

Zipora Yablonka-Reuveni, Ph.D.

(RG) Pericytes as means for cell-based therapy to treat muscular dystrophy

\$ 128,591 07/01/2009 - 06/30/2010 (Year 1)

\$ 131,781 07/01/2010 - 06/30/2011 (Year 2)

\$ 135,069 07/01/2011 - 06/30/2012 (Year 3)

Summary. One approach to treating Duchenne muscular dystrophy and other muscle wasting disorders is to identify expandable sources of cells that possess or can adapt a skeletal myogenic fate and introduce the cells to patients to restore muscle mass and repair capacity. Such cell-based approaches require efficient cell delivery to many muscles. Thus far, *in vivo* dispensing of donor myoblasts (cells that naturally form muscle fibers) has not proven practical. Recent findings have brought forward the possible use of pericytes (microvascular-associated cells) for cell-based therapy in DMD. However, characterization of these cells was based on their properties after expansion in culture and therefore their true nature is unknown. A better understanding of the skeletal myogenic potential of bona fide pericytes is required so that the phenomenon can be studied in a reproducible manner. In the proposed project we will utilize mice that express specific tracers in pericytes and investigate pericyte contribution to skeletal myogenesis in several mouse models of muscular dystrophy. The anticipated outcome of the proposed studies will contribute new insights for devising treatments of muscular dystrophy disorders in human patients.

2012 ACTIVE GRANTS

AUSTRALIA

Clayton - Monash University

Peter Currie, PhD

(RG) Small molecule screening in a zebrafish model of Duchenne Muscular Dystrophy

\$ 125,000 08/01/2011 - 07/31/2012 (Year 1)

\$ 125,000 08/01/2012 - 07/31/2013 (Year 2)

\$ 125,000 08/01/2013 - 07/31/2014 (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 utilise 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 Nicholson, M.D., PhD

(RG) Determining the Pathogenic Effects of ATP7A Mutations in Distal Motor Neuropathy

\$ 140,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 140,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 140,000 02/01/2014 - 01/31/2015 (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.

Melbourne - The University of Melbourne

Gordon Lynch, Ph.D.

(RG) Modulating IGF:IGFBP signaling to improve muscle function in muscular dystrophy

\$ 125,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 125,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 125,000 07/01/2012 - 06/30/2013 (Year 3)

Summary: Muscle wasting and weakness are major symptoms of many muscular 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, 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.

Nedlands - The University of Western Australia

Kristen Nowak, Ph.D.

(RG) Discovering modifier genes for neuromuscular disease severity

\$ 162,692 08/01/2011 - 07/31/2012 (Year 1)

\$ 166,399 08/01/2012 - 07/31/2013 (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.

Parkville - Murdoch Childrens Research Institute

Shireen Lamande, Ph.D.

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

\$ 126,735 02/01/2011 - 01/31/2012 (Year 1)

\$ 124,861 02/01/2012 - 01/31/2013 (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 02/01/2011 - 01/31/2012 (Year 1)

\$ 138,750 02/01/2012 - 01/31/2013 (Year 2)

\$ 138,750 02/01/2013 - 01/31/2014 (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 Zacharin, F.R.A.C.P

(RG) Clinical trial of Zoledronic acid in children and adolescents with Duchenne(DMD)

\$	138,171	08/01/2011 - 07/31/2012	(Year 1)
\$	129,850	08/01/2012 - 07/31/2013	(Year 2)

Summary: Chronic, progressive immobilization is an inevitable consequence of Duchenne muscular 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

Perth - The University of Western Australia

Steve Wilton, Ph.D

(RG) Preclinical assessment of splice switching oligomers

\$	122,700	07/01/2010 - 06/30/2011	(Year 1)
\$	122,700	07/01/2011 - 06/30/2012	(Year 2)
\$	122,700	07/01/2012 - 06/30/2013	(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 (2OMe 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.

Sydney - The University of Sydney

Des Richardson, Ph.D., D.Sc.

(RG) Development of Iron Complexes for the Treatment of Friedreich's Ataxia

\$	150,000	08/01/2011 - 07/31/2012	(Year 1)
\$	150,000	08/01/2012 - 07/31/2013	(Year 2)
\$	150,000	08/01/2013 - 07/31/2014	(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

Antwerpen - Flanders Institute for Biotechnology and University of Antwerp

Albena Jordanova, PhD

(RG) Identification of molecular players and drug targets for CMT neuropathies

\$	94,210	07/01/2010 - 06/30/2011	(Year 1)
\$	94,210	07/01/2011 - 06/30/2012	(Year 2)
\$	94,210	07/01/2012 - 06/30/2013	(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 synthetase. 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	02/01/2012 - 01/31/2013	(Year 1)
\$	141,984	02/01/2013 - 01/31/2014	(Year 2)

\$ 141,984 02/01/2014 - 01/31/2015 (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

Rashmi Kothary, Ph.D.

(RG) Defining a role for SMN protein in actin dynamics and SMA pathogenesis

\$ 116,160 07/01/2009 – 06/30/2010 (Year 1)

\$ 116,160 07/01/2010 - 06/30/2011 (Year 2)

\$ 116,160 07/01/2011 - 06/30/2012 (Year 3)

Summary: SMA is a children's disorder that involves degeneration of motor neurons and subsequent wasting of proximal muscles. In this project, we take advantage of cell culture and mouse models to study the contribution of actin cytoskeleton (a scaffold within the cell) disruption to SMA pathogenesis. These efforts will improve our understanding of SMN in motor neurons in the context of the SMA disease and to reveal important steps in the pathophysiology of SMA.

Lynn Megeney, Ph.D.

(RG) The Role of Caspase 3 in Satellite Cell Self Renewal

\$ 124,260 01/01/2010 - 12/31/2010 (Year 1)

\$ 124,260 01/01/2011 - 12/31/2011 (Year 2)

\$ 124,260 01/01/2012 - 12/31/2012 (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 satellite cell maturation into muscle fibers are well understood, we have little understanding of what controls the initial activation or renewal of these stem cells. 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 maturation process to muscle. We propose

to investigate the mechanisms by which caspase 3 impairs muscle stem cell self renewal.

Michael Rudnicki, PhD

(RG)	Molecular Regulation of Satellite Cell Function		
\$	125,000	08/01/2011 - 07/31/2012	(Year 1)
\$	125,000	08/01/2012 - 07/31/2013	(Year 2)
\$	125,000	08/01/2013 - 07/31/2014	(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

Bernard Jasmin, PhD

(RG)	Impact of exercise mimetics on the dystrophic pathology in the mdx mouse		
\$	120,000	07/01/2010 - 06/30/2011	(Year 1)
\$	120,000	07/01/2011 - 06/30/2012	(Year 2)
\$	120,000	07/01/2012 - 06/30/2013	(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	02/01/2012 - 01/31/2013	(Year 1)
\$	60,000	02/01/2013 - 01/31/2014	(Year 2)
\$	60,000	02/01/2014 - 01/31/2015	(Year 3)

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

Aymeric Ravel-Chapuis, Ph.D.

(DG) Role of the RNA-binding protein Staufen1 in Myotonic Dystrophy type1

\$ 59,950 07/01/2010 - 06/30/2011 (Year 1)

\$ 59,950 07/01/2011 - 06/30/2012 (Year 2)

\$ 59,950 07/01/2012 - 06/30/2013 (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.

Ilona Skerjanc, Ph.D.

(RG) Enhanced muscle repair with human embryonic stem cells

\$ 140,604 02/01/2012 - 01/31/2013 (Year 1)

\$ 139,883 02/01/2013 - 01/31/2014 (Year 2)

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.

Nadine Wiper-Bergeron, Ph.D

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

\$ 105,165 02/01/2012 - 01/31/2013 (Year 1)

\$ 103,604 02/01/2013 - 01/31/2014 (Year 2)

\$ 103,653 02/01/2014 - 01/31/2015 (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.

Québec

Montreal - Centre hospitalier de l'Université de Montreal (CHUM) Research Center

Guy Rouleau, M.D., Ph.D.

(RG) Whole exome sequencing in patients with familial ALS

\$ 135,653 01/01/2010 - 12/31/2010 (Year 1)

\$ 135,653 01/01/2011 - 12/31/2011 (Year 2)

\$ 135,653 01/01/2012 - 12/31/2012 (Year 3)

Summary: While the discovery of several genes has led to significant new insights into the cause of ALS, both the basic pathogenic mechanism and the genetic basis of most cases remain unknown. Therefore, it is still necessary to identify additional ALS-causing genes. To accomplish this, we will use the more recent and powerful generation of DNA sequencing technologies to resequence the entire coding genome "all the coding genes," or "exome," in a collection of 32 familial ALS patients (and unaffected relatives) that were selected from our biggest and best clinically characterized families segregating ALS. This will enable us to identify novel potentially causative ALS genes. Any genes

shown to underlie familial ALS will subsequently be screened in additional familial and sporadic ALS cases to confirm and establish their genuine implication.

Montreal - McGill University

Josephine Nalbantoglu, Ph.D.

(RG) Artificial zinc fingers targeting the human utrophin promoter

\$ 104,390 07/01/2010 - 06/30/2011 (Year 1)

\$ 104,390 07/01/2011 - 06/30/2012 (Year 2)

\$ 104,390 07/01/2012 - 06/30/2013 (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.

Quebec - CHUL Research Center

Jasna Kriz, Ph.D.

(RG) Live Imaging of ALS Pathogenesis and Therapeutic Efficacy

\$ 148,362 08/01/2011 - 07/31/2012 (Year 1)

\$ 148,362 08/01/2012 - 07/31/2013 (Year 2)

\$ 148,362 08/01/2013 - 07/31/2014 (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.

Quebec - Université Laval

Francois Berthod, Ph.D.

(RG) Development of a human tissue-engineered model of the spinal cord to study ALS

\$ 115,698 02/01/2011 - 01/31/2012 (Year 1)

\$ 115,698 02/01/2012 - 01/31/2013 (Year 2)

\$ 115,698 02/01/2013 - 01/31/2014 (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 risk factor and modifier on disease onset for ALS

\$	115,000	07/01/2010 - 06/30/2011	(Year 1)
\$	115,000	07/01/2011 - 06/30/2012	(Year 2)
\$	115,000	07/01/2012 - 06/30/2013	(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 - University of Chile

Claudio Hetz, Ph.D

(RG) Role of ER stress in SOD1 wild-type misfolding: A model for sporadic ALS?

\$	72,500	02/01/2012 - 01/31/2013	(Year 1)
\$	72,500	02/01/2013 - 01/31/2014	(Year 2)
\$	72,500	02/01/2014 - 01/31/2015	(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	08/01/2011 - 07/31/2012	(Year 1)
\$	125,000	08/01/2012 - 07/31/2013	(Year 2)
\$	116,210	08/01/2013 - 07/31/2014	(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.

FRANCE

Evry - Genethon

Isabelle Richard,

(RRG) Restricted Funds for Research - McCaw Fund

\$	6,721	11/1/2011 – 10/31/2012	(Year 1)
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Illkirch - CERBM GIE

Jocelyn Laporte, Ph.D.

(RG) Identification of novel genes mutated in centronuclear myopathies

\$	119,750	02/01/2011 - 01/31/2012	(Year 1)
\$	119,750	02/01/2012 - 01/31/2013	(Year 2)
\$	101,750	02/01/2013 - 01/31/2014	(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.

Paris - Institut de Cerveau et de la Moelle

Edor Kabashi, Ph.D.

(DG) Functional Characterization of TDP-43 Mutations in Amyotrophic Lateral Sclerosis

\$	60,000	07/01/2009 – 06/30/2010	(Year 1)
\$	60,000	07/01/2010 - 06/30/2011	(Year 2)
\$	60,000	07/01/2011 - 06/30/2012	(Year 3)

Summary: The genetic cause of only 1-2% of all ALS cases is accounted for by mutations in the SOD1 gene. Consequently the identification of novel ALS-causative genes remains imperative. TDP-43 has been demonstrated to be a major component of the protein aggregates seen in ALS patients but no mutation in the TARDBP gene that encodes TDP-43 protein had been identified. Our group and several other research teams have recently identified and genetically validated mutations of TARDBP in sporadic and familial ALS cases. This suggests that mutant TDP-43 is the sole cause of ALS in a subset of patients. This project will help to determine and understand the negative impact of TARDBP mutations in ALS and motor neuron degeneration. In order to understand the toxic aspects of mutant TDP-43 I am currently establishing appropriate models in cells (motor neurons from spinal cord cultures) and living organisms (zebrafish). Functional characterization of mutant TDP-43 in these models will help to determine what the molecular partners of mutant TDP-43 are as well as the role this protein normally plays in motor neuron biology. Finally, these novel models will be used to screen a large number of pharmaceutical compounds that should directly open avenues for the development of therapies that could delay or prevent disease onset and progression in all ALS patients.

GREECE

Athens - HELLENIC PASTEUR INSTITUTE

Socrates Tzartos, Ph.D.

(RG) Antigen-specific therapeutic autoantibody depletion in myasthenia gravis

\$ 116,700 01/01/2010 - 12/31/2010 (Year 1)

\$ 116,700 01/01/2011 - 12/31/2011 (Year 2)

\$ 116,700 01/01/2012 - 12/31/2012 (Year 3)

Summary: Myasthenia gravis (MG) is a neuromuscular disease caused mainly by an autoimmune response against the muscle nicotinic acetylcholine receptor (AChR) which interferes with neuromuscular transmission. In MG patients, autoantibodies lead to loss of AChRs, resulting in impaired neuromuscular transmission reflected by weakness and fatigability of voluntary muscles. Current treatments are non-specific and most are associated with severe side-effects. The extensive and valuable information available on MG makes this disease a most appropriate target for the development of an antigen-specific therapy. Our main aim is to develop an MG-specific therapeutic approach aimed at the selective depletion of the Abs from patients' blood and the return of the cleared blood to the patient. This project is based on our very encouraging results for the clearance of MG sera using immobilized recombinant extracellular domains (ECDs) of the five AChR subunits, and has three specific aims: I) To increase the immunoadsorption efficiency of the ECDs by the expression of native-like pentamers assembled from the five ECDs. II) To investigate the in vivo effectiveness of Ab depletion by studying the pathogenicity of the untreated sera, Ab-depleted sera, and the purified Abs in rodents. III) To scale up the immunoadsorption procedure and initiate preliminary clinical trials.

HONG KONG

Kowloon - The Hong Kong University of Science and Technology

Chi Wai Lee, Ph.D.

(DG) The Mechanism of Postsynaptic AChR Endocytosis in Myasthenia Gravis Pathogenesis

\$ 60,000 02/01/2011 - 01/31/2012 (Year 1)

\$ 60,000 02/01/2012 - 01/31/2013 (Year 2)

\$ 60,000 02/01/2013 - 01/31/2014 (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.

ISRAEL

Petha Tikva - Tel - Aviv University

Daniel Offen, Ph.D.

(RG) Myogenic cells – possible application in autologous cell and gene therapy of ALS

\$ 119,900 07/01/2010 - 06/30/2011 (Year 1)

\$ 119,900 07/01/2011 - 06/30/2012 (Year 2)

\$ 119,900 07/01/2012 - 06/30/2013 (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

Rehovot - Weizmann Institute of Science

Ben-Zion Shilo, PH.D

(RG) WASp-based Actin Polymerization Promotes Myoblast Fusion in Drosophila and Mice

\$ 100,000 07/01/2009 – 06/30/2010 (Year 1)

\$ 100,000 07/01/2010 - 06/30/2011 (Year 2)

\$ 100,000 07/01/2011 - 06/30/2012 (Year 3)

Summary: Skeletal muscles are formed by the fusion of multiple individual muscle cells (myoblasts), generating a single large muscle fiber. Much remains unknown about the molecular mechanisms governing this fundamental process, which is essential for construction of healthy muscles, capable of performing diverse and often strenuous tasks. Research in model organisms has enabled the study of a wide variety of biological issues related to human health, by application of genetic approaches. We plan to follow this route, and study myoblast fusion in two prominent model systems, the Drosophila fruit fly, and mice. Adult fly muscles bear considerable morphological similarities to human muscles, but the mechanisms involved in their construction are not well understood. We will use the detailed description available for myoblast fusion in the fly embryo, together with recently developed, powerful genetic tools, to guide us in establishing molecular models for adult fly muscle formation. In parallel, we will build upon our discovery that elements essential for construction of muscle fibers in Drosophila, perform similar functions in mice. We now plan to thoroughly characterize the manner by which these elements contribute to mouse myoblast fusion. Understanding the normal mechanism of muscle cell fusion is expected to facilitate the design of methods for correction of muscle impairments in humans, such as promoting fusion of satellite myoblasts with damaged or defective muscle fibers.

ITALY

Genova - Fondazione Istituto Italiano di Tecnologia

Maria Pennuto, Ph.D.

(RG)	PKA signaling in SBMA pathogenesis		
\$	110,000	08/01/2011 - 07/31/2012	(Year 1)
\$	110,000	08/01/2012 - 07/31/2013	(Year 2)
\$	110,000	08/01/2013 - 07/31/2014	(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

(RG)	Role of Sam68 in the regulation of SMN2 alternative splicing in SMA cells		
\$	73,000	02/01/2011 - 01/31/2012	(Year 1)
\$	73,000	02/01/2012 - 01/31/2013	(Year 2)
\$	73,000	02/01/2013 - 01/31/2014	(Year 3)

Summary: Human genes contain introns that need to be excised from the mRNA through a process 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 Noordermeer, Ph.D.

(RG) Elucidating the Synaptic Roles of Dystrophin

\$	97,992	08/01/2011 - 07/31/2012	(Year 1)
\$	90,289	08/01/2012 - 07/31/2013	(Year 2)
\$	90,289	08/01/2013 - 07/31/2014	(Year 3)

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 van der Maarel, Ph.D.

(RG) AONs to modulate the transcriptional landscape of D4Z4

\$	99,323	04/01/2010 – 03/31/2011	(Year 1)
\$	99,746	04/01/2011 – 03/31/2012	(Year 2)

Summary: Facioscapulohumeral muscular dystrophy (FSHD) is caused by contractions of the D4Z4 repeat on the 4A161 permissive haplotype of the subtelomere of chromosome 4q. Recent evidence from our laboratory and others points towards a critical role of the most distal D4Z4 repeat unit. A complex transcriptional profile emanates from this repeat unit which is disturbed in FSHD. In the current proposal we aim to modulate these aberrant transcripts in FSHD by use of antisense oligonucleotides (AONs). AONs are powerful molecules that can affect the splicing of target genes by specific binding to nascent RNA and have been shown to have therapeutic efficacy in a number of diseases. AONs will be designed and tested in vitro and in vivo on immortalized FSHD myoblasts and in a transgenic mouse model carrying D4Z4 repeats. Both models show D4Z4 transcripts that have the potential to cause FSHD. As there is already extensive experience with the use of AONs in humans, in case of potential therapeutic efficacy, it is expected that such AONs can be readily applied in clinical trials for FSHD.

Silvere van der Maarel, Ph.D.

(RG) Molecular pathophysiology of FSHD2

\$	131,560	02/01/2012 - 01/31/2013	(Year 1)
\$	124,910	02/01/2013 – 01/31/2014	(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 commonalities 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.

Silvere van der Maarel, Ph.D.

(SGNEW) FSH Society FSHD International Research Consortium Workshop

\$ 5,000 11/01/2011 – 11/30/2011 (Year 1)

Summary: The FSHD International Research Consortium workshop brings together clinicians, scientists, patient representatives and policy makers to discuss the latest developments in FSHD. For seventeen years this workshop has provided the FSHD community with a forum to present and discuss new developments, reinforce collaborative efforts, facilitate new initiatives, and coordinate research and clinical activities. In the past year, there have been seminal breakthrough publications on the molecular pathophysiology of FSHD and use of RNA interference on animal models of FSHD. With numerous developments, large sets of data, more availability of biomaterials from multiple large research initiatives for FSHD, a meeting for dissemination of new ideas, discussion of new strategies, collaboration and coordination is needed more than ever. To facilitate participation from the U.S., Europe and globally, this year the workshop will be held near Boston in Watertown, Massachusetts to pursue five goals: 1) Given the recent exciting breakthrough with the potential to ameliorate the onset of FSHD it is critical for the community to convene and discuss new data and advances in FSHD; 2) to discuss strategies to verify and independently corroborate the findings; 3) to discuss focusing efforts and resources in the preclinical gap and translational phase of research; 4) to improve diagnostic techniques and criteria for FSHD, and; 5) to consider and evaluate with industry new and existing therapies.

Nijmegen - Radboud University Nijmegen Medical Centre

Berend Wieringa, Ph.D., Prof.

(RG) Oligonucleotide-mediated silencing of (CUG)_n RNA stress in Myotonic Dystrophy

\$ 134,261 07/01/2009 – 06/30/2010 (Year 1)

\$ 131,661 07/01/2010 - 06/30/2011 (Year 2)

\$ 130,061 07/01/2011 - 06/30/2012 (Year 3)

Summary: The main burden of myotonic dystrophy type 1 (DM1) is the continuous worsening of muscle, brain and cardiac problems. This progressive nature can be explained by (i) ongoing somatic expansion of the (CTG•CAG)_n repeat in the DMPK gene

and (ii) cellular toxicity of abnormal gene transcripts causing misregulation of normal cellular programming. One may expect that treatment aimed at reducing these RNA-based effects will alleviate the main problems of DM1. In this proposal, a recently developed strategy based on selective silencing of toxic DMPK mRNA will be explored (manuscript under review; presented at IDMC-6, 2007). We have demonstrated that a fully-modified 2'-O-methyl-phosphorothioate (CAG)_n oligonucleotide, complementary to the (CUG)_n tract, efficiently eliminates expanded DMPK transcripts in myoblasts derived from mouse models and patients. In addition, reversal of nuclear RNP aggregation and RNA-mis-splicing, typical DM1 disease hallmarks, was obtained after oligo administration in muscles of DM1 mice *in vivo*. We will bring these findings a step closer to the clinic via a detailed study of the pathobiological-reversal effects and working mechanism(s) of (CAG) type oligos *in vitro* in multiple DM1-relevant cell types, in skeletal muscle cells and newly-to-develop DM1 cardiac and brain cell models. The work will be performed in parallel to *in vivo* mouse studies, with the ultimate aim to better understand oligo effects and safety of use in future clinical applications.

SPAIN

Barcelona - Universitat Pompeu Fabra

Pura Munoz-Canoves, Ph.D.

(RG) Cellular Mechanisms Of Fibrosis Development In Muscular Dystrophies

\$ 130,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 130,000 02/01/2013 - 01/31/2014 (Year 2)

\$ 130,000 02/01/2014 - 01/31/2015 (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 - Lund University

Madeleine Durbeej-Hjalt, Ph.D.

(RG) Proteasome and autophagy inhibition in congenital muscular dystrophy

\$ 147,341 02/01/2011 - 01/31/2012 (Year 1)

\$ 147,341 02/01/2012 - 01/31/2013 (Year 2)

\$ 147,341 02/01/2013 - 01/31/2014 (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

London - Institute of Child Health, University College London

Francesco Muntoni, M.D.

(RG) Uncovering the role of mitochondria in the pathogenesis of core myopathies

\$ 125,000 07/01/2010 - 06/30/2011 (Year 1)

\$ 125,000 07/01/2011 - 06/30/2012 (Year 2)

\$ 125,000 07/01/2012 - 06/30/2013 (Year 3)

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 Brown, PhD

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

\$ 118,946 08/01/2011 - 07/31/2012 (Year 1)

\$ 118,946 08/01/2012 - 07/31/2013 (Year 2)

\$ 118,946 08/01/2013 - 07/31/2014 (Year 3)

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.

Oswestry - Keele University

Glenn Morris, D. Phil.

(RIG) The MDA Monoclonal Antibody Resource for Neuromuscular Disease

\$	127,985	10/01/2010 - 09/30/2011	(Year 1)
\$	122,744	10/01/2011 - 09/30/2012	(Year 2)
\$	124,696	10/01/2012 - 09/30/2013	(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.

Oxford - University of Oxford

Kay Davies, MA, Ph.D.,

(RG) Approaches to therapy for DMD

\$	165,814	01/01/2010 - 12/31/2010	(Year 1)
\$	107,883	01/01/2011 - 12/31/2011	(Year 2)
\$	107,883	01/01/2012 - 12/31/2012	(Year 3)

Summary: Our long term objective is to develop an effective therapy for Duchenne muscular dystrophy. We are approaching this in two ways. The first is to increase the amount of a protein called utrophin in muscle cells. Utrophin is very similar to dystrophin and should be able to functionally replace the missing dystrophin in patients. We have shown that this is possible in the mouse and dog models of the disease and we have one drug, C1100, which is proceeding to clinical trial. More follow up compounds need to be developed to improve on current drug effectiveness. The second strategy is called exon-skipping, whereby antisense oligonucleotides are used to trick the muscle cell into skipping over the deletion in patients to produce a functional but shorter dystrophin. The principle of exon-skipping has been demonstrated to work in early human trials by direct injection into muscle. We intend to maximize the efficiency of this technique using different constructs in viruses so that it can be applied clinically to more patients. In the longer term, a combination of these approaches may be needed to treat DMD patients.

Kay Davies, MA, Ph.D.,

(RG) Screening for drugs to increase utrophin levels in DMD

\$ 130,000 02/01/2012 - 01/31/2013 (Year 1)

\$ 130,000 02/01/2013 - 01/31/2014 (Year 2)

Summary. Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disease caused by the absence of the large protein 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.