AUSTRALIA

Clayton - Monash University

Peter David Currie PhD

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

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

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

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

$140,000.00 2/1/2013 1/31/2014 Year 2

$140,000.00 2/1/2014 1/31/2015 Year 3

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

Murdoch - Murdoch University

Steve D Wilton Ph.D

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

$100,000.00 8/1/2013 7/31/2014 Year 1

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

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

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

Parkville - The University of Melbourne

Gordon Stuart Lynch Ph.D.
**RG** Therapeutic potential of heat shock protein 72 induction in muscular dystrophy

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**Summary**

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

**Sydney - The University of Sydney**

**Joshua Burns Ph.D.**

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

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**Summary**

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

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

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

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**Summary**

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

**BELGIUM**

**Gent - VIB vzw**

**Ludo Van Den Bosch Ph.D.**

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

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**Summary**

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

CANADA

NOVA SCOTIA

Halifax - AGADA Biosciences

Kitipong Uaesoontrachoon PhD

RIG Murine Preclinical Center for Neuromuscular Diseases (MPCNMD)

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

ONTARIO

Ottawa - Children's Hospital of Eastern Ontario Research Institute Inc

Robert Korneluk Ph.D

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.

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

**Lynn Megeney Ph.D.**

**RG** Caspase 3 Limits the Renewal of Activated Satellite Cells  
$100,000.00$ 8/1/2013 7/31/2014 Year 1  
$100,000.00$ 8/1/2014 7/31/2015 Year 2  
$100,000.00$ 8/1/2015 7/31/2016 Year 3  

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

**Michael A. Rudnicki PhD**

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

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

**Ottawa - University of Ottawa**

**Bernard Jasmin PhD**

**RG** The RNA-binding protein Staufen1 as a target for novel therapies for DM1  
$84,600.00$ 5/1/2014 4/30/2015 Year 1  
$84,600.00$ 5/1/2015 4/30/2016 Year 2  
$84,600.00$ 5/1/2016 4/30/2017 Year 3  

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

**Vladimir Ljubicic Ph.D.**

**DG** Dissecting the mechanisms underlying the benefits of novel therapeutics for DMD  
$60,000.00$ 2/1/2013 1/31/2014 Year 2  
$60,000.00$ 2/1/2014 1/31/2015 Year 3
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.

Nadine Wiper-Bergeron Ph.D.

Summar

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 de Recherche du Centre hospitalier de l'Université de Montreal

Alex Parker Ph.D

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

$103,604.00 2/1/2013 1/31/2014 Year 2

$103,653.00 2/1/2014 1/31/2015 Year 3

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

Montreal - CHUM Research Center

Christine Vande Velde Ph.D.

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

$75,100.00 8/1/2013 7/31/2014 Year 2

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

Summary TDP-43 is a recently identified gene associated with Amyotrophic Lateral Sclerosis (ALS). Very little is known about how mutations in TDP-43 cause ALS. My goal is to better understand both the normal biological role of TDP-43 as well as the mechanism of neuronal toxicity in the pathological condition. For neurodegenerative diseases, the path from genetic mutation to neuronal dysfunction and cell death is complex and in humans takes many decades. Thus, convenient systems are needed to pursue the manipulation of neuronal survival at a comprehensive, genome and organism-wide level. Using invertebrates like C. elegans, to model human disorders has emerged as a useful strategy in the neurodegeneration field. I will use worm deletion mutants of TDP-43 to learn more about the gene’s normal biological roles in the cellular stress response. This information may shed light on disease mechanisms in ALS patients. I have also created transgenic worms that express mutant human TDP-43 in motor neurons that I will use to discover genetic mechanisms to reduce neurodegeneration. My preliminary data suggests a role for the unfolded protein response in TDP-43 & FUS motor neuron toxicity. Further understanding the role of TDP-43 in stress response signaling may aid drug discovery efforts to arrest disease progression and provide a better quality of life for ALS patients.

Christine Vande Velde Ph.D.

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

$119,414.00 8/1/2013 7/31/2014 Year 2

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

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

Montreal - McGill University

Heather D. Durham Ph.D.

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

$119,322.00 2/1/2013 1/31/2014 Year 1
$118,611.00 2/1/2014 1/31/2015 Year 2
$118,003.00 2/1/2015 1/31/2016 Year 3

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

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

Benoit Coulombe Ph.D.

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

$125,689.00 2/1/2013 1/31/2014 Year 1
$125,689.00 2/1/2014 1/31/2015 Year 2
$125,689.00 2/1/2015 1/31/2016 Year 3

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

Quebec - CHUL Research Center

Jasna Kriz Ph.D.

RG Live Imaging of ALS Pathogenesis and Therapeutic Efficacy

$148,362.00 8/1/2013 7/31/2014 Year 3

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

$116,210.00  8/1/2013  12/31/2014  Year 3

Summary

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

CYPRUS

Nicosia - CING - The Cyprus Institute of Neurology and Genetics

Kleopas A. Kleopa M.D.

RG

Developing gene therapy for inherited neuropathy

$91,663.00  8/1/2013  7/31/2014  Year 1

$93,287.00  8/1/2014  7/31/2015  Year 2

$95,995.00  8/1/2015  7/31/2016  Year 3

Summary

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

FRANCE
Ilkirk - CERBM GIE
Jocelyn Laporte Ph.D. Molecular Biology

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

GREECE
Athens - HELLENIC PASTEUR INSTITUTE
Socrates J Tzartos Ph.D.

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

ISRAEL
Jerusalem - Hebrew University of Jerusalem
Yosef Gruenbaum Ph.D.

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

ITALY

Genova - Fondazione Istituto Italiano di Tecnologia

Maria Pennuto Ph.D.

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.

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

Nicoletta Filigheddu Ph.D.

Summary

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

NETHERLANDS

Leiden - LUMC

Jasprina N. Noordermeer Ph.D.

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.

**SPAIN**

**Barcelona - Universitat Pompeu Fabra**

Pura Munoz-Canoves Ph.D.

**CELLULAR MECHANISMS OF FIBROSIS DEVELOPMENT IN MUSCULAR DYSTROPHIES**

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**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.

**UNITED KINGDOM**

**Edinburgh - University of Edinburgh**

Lyndsay Murray Ph.D.

**Expression profiling of differentially vulnerable motor neurons in SMA**

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

**GLASGOW - University of Glasgow**

Darren G. Monckton Ph.D.

**Next generation sequencing approaches to genotyping in myotonic dystrophy type 1**

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

**London - Royal Veterinary College**

**Susan Carol Brown PhD**

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

$118,946.00  8/1/2013  7/31/2014  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.

**Oxford - University of Oxford**

**Kay Elizabeth Davies MA, Ph.D.,**

RG  Utrophin upregulation for treatment of DMD

$103,783.00  2/1/2013  1/31/2014  Year 1

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

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

**Portsmouth - University of Portsmouth**

**Darek Gorecki Ph.D.**

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

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

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

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

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

**UNITED STATES**

**ALABAMA**

**Birmingham - Southern Research Institute**

**Maurizio Grimaldi M.D.**
Development of small molecules active at disease onset in ALS

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

[Birmingham - The University of Alabama at Birmingham]

Marek Napierala Ph.D.

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

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

[ARIZONA]

Phoenix - The Translational Genomics Research Institute

Lisa Baumbach Ph.D.

Identification of New Disease Genes for Infantile Lower Motor Neuron Diseases

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, UBE1 (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/colllected 20 new cases of putative XL-SMA, and are completing UBE1 mutation screening. Despite numerous phenotypic similarities of these patients with our previously reported UBE1 mutation-positive XL-SMA cases, no new UBE1 mutations have been detected. This raises strong suspicion of yet- to- be identified mutations and disease genes. Our long-term goal is to apply knowledge gained from XL-SMA disease gene discovery (and these investigations) to
other related forms of infantile lower motor neuron disease (ILMD), thus improving prenatal and antenatal disease detection, as well as eventual therapeutic strategies. The goal of this application is to use several contemporary experimental approaches to identify novel disease genes for ILMD in this unique patient cohort.

**Tucson - Arizona Board of Regents, University of Arizona**

**Henk Granzier PhD**

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**Summary**

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

**Archi Joardar**

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**Summary**

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurological disorder that leads to paralysis and death. The pathological features of this devastating disorder include motor neuron loss and muscle atrophy. With the recent identification of cellular inclusions containing TDP-43 protein plus the discovery of mutations in TDP-43 in patients, this protein has emerged as a common denominator in a significant fraction of ALS cases. We have found that human TDP-43 carrying mutations identical to those found in ALS patients, when expressed in fruit fly motor neurons, leads to neuroanatomical and locomotor defects that mimic clinical manifestations of the human disease. Testing this Drosophila model of ALS against a large panel of FDA-approved drugs, we identified some that alleviated neurotoxicity of human TDP-43 in the fly. Here we propose to further develop a subset of those compounds, which target a nuclear protein involved in cellular metabolism. We will use genetic interactions to determine what aspects of TDP-43 neurotoxicity are mediated by our newly discovered candidate target. These whole organism data will be translated to mammalian systems using in vitro assays in a collaboration with Sanofi. Through this collaborative approach, involving academic research in an animal model of ALS combined with proven drug discovery tools in an industry setting, we are well positioned to discover novel therapeutic targets and strategies for ALS that might eventually lead.

**Daniela Zarnescu Ph.D.**

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**Summary**

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

CALIFORNIA
Davis - The Regents of the University of California (University of California Davis)
David Paul Richman M.D.
RG Pathogenesis of Anti-MuSK Myasthenia
$137,500.00 2/1/2013 1/31/2014 Year 1
$137,500.00 2/1/2014 1/31/2015 Year 2
$137,500.00 2/1/2015 1/31/2016 Year 3

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

La Jolla - Ludwig Institute for Cancer Research Ltd
Don Cleveland Ph.D.
RG Determining the contribution of mitochondrial dysfunction in ALS pathogenesis
$133,981.00 8/1/2013 7/31/2014 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.

Clotilde Lagier-Tourenne M.D., Ph.D.
DG Determining RNA metabolism alterations in Amyotrophic Lateral Sclerosis
$60,000.00 8/1/2013 7/31/2014 Year 3

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

Alessandra Sacco Ph.D.

**In utero and neonatal stem cell therapy for Duchenne Muscular Dystrophy**

$148,777.00 8/1/2013 7/31/2014 Year 3

**Summary**

Duchenne muscular dystrophy (DMD) starts inevitably at birth, and by the time patients are diagnosed and begin their treatment, irreversible damage has already accumulated in skeletal muscle and its function is permanently lost. This aspect poses a major hurdle to cell-based therapies in young adults, as they face the challenge of rescuing permanently damaged tissue. We hypothesize that therapeutic intervention during fetal and neonatal stages will overcome this roadblock, as no major injury has been inflicted to the tissue, dissemination of cells throughout developing muscles will be more efficient, and immune tolerance towards donor cells can be induced. Accordingly, in this project we will develop in utero and neonatal muscle stem cell (MuSC) transplantation strategies in dystrophic animal models and evaluate cell survival, proliferation and migration of donor cells within host muscles, induction of immune tolerance and finally assess the therapeutic improvement in muscle function. To this aim, we will employ three powerful tools we recently developed: (1) An approach to prospectively isolate adult MuSC, (2) A noninvasive bioluminescence imaging assay to monitor the dynamic behavior of MuSC in vivo, and (3) A novel mouse model of DMD, mdx mice lacking telomerase activity, that closely mimics the clinical progression in humans, ideally suited for testing potential therapies.

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

Adam Jeffrey Engler Ph.D.

**Mechanically programmed adipose-derived stem cells to treat muscular dystrophy**

$130,000.00 8/1/2013 7/31/2014 Year 2

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

**Summary**

The major challenge of restoring muscle contraction to patients with muscular dystrophy has been to deliver cells that can overcome the fibrotic, stiff cell niche of the degenerated muscle, avoid converting into intramuscular fat, and fuse with muscle fibers. While several cell sources have been proposed, most adult stem cell sources are not abundant for clinically viable treatment, cannot fuse into dystrophic muscle, or cannot restore function. However, we have mechanically induced adipose-derived stem cells (ASCs) to become muscle, and they can maintain their fused muscle state in dystrophic muscle-like environments in vitro. In this project, we will first understand the differences between how ASCs and other cell sources, including satellite cells and intramuscular fat, sense and respond to stiffness, which enables ASC-derived muscle and intramuscular fat to retain their fates despite the presence of a stiff environment instructing the cells to become other tissues. We will then assess their fusion potential with host animals and determine their ability to form ex vivo innervated tissue constructs in bioreactor cultures that mimic dystrophic muscle. Finally, we will perform intramuscular injections of ASC-derived myotubes and assess engraftment, dystrophin expression, and restoration of degenerated muscle function. Successful validation of functional muscle restoration using ASC-derived muscle will lead to larger animal studies and potential clinical translation.

Masahiko Hoshijima M.D., Ph.D.

**Genetic treatment of cardio-respiratory failure in muscular dystrophy**

$122,462.00 2/1/2013 7/31/2014 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.

**SBMA motor neuron degeneration: molecular basis and therapy**

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

La Jolla - The Scripps Research Institute
Matthew Disney Ph.D.
RG Identification & Optimization of Small Molecules Targeting r(CCUG)exp in DM2
$120,908.00 2/1/2013 1/31/2014 Year 1
$120,908.00 2/1/2014 1/31/2015 Year 2
$120,908.00 2/1/2015 1/31/2016 Year 3

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

David Samuel Gokhin Ph.D.
DG Structure, Regulation, and Function of Gamma-Actin in the Sarcoplasmic Reticulum
$60,000.00 8/1/2013 7/31/2014 Year 2
$60,000.00 8/1/2014 7/31/2015 Year 3

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

Sunitha Rangaraju Ph.D
DG Improving ALS phenotypes by targeting aging pathways
$60,000.00 2/1/2013 1/31/2014 Year 1
$60,000.00 2/1/2014 1/31/2015 Year 2
$60,000.00 2/1/2015 1/31/2016 Year 3
Our aim is to identify molecules that could be developed into therapeutics for amyotrophic lateral sclerosis (ALS). ALS is an age-related neuromuscular disease whose progression occurs with aging and is thought to be driven by the aberrant aggregation of certain proteins, which then leads to the collapse of protein homeostasis. We reasoned that, molecules that delay aging may prove as effective therapeutics for ALS. We previously screened over 89,000 molecules for those that delay aging and extend lifespan of C. elegans, a small worm that is widely used to study aging. We identified over 100 molecules that extend lifespan, which I am currently testing on a C. elegans model of ALS, to identify potential therapeutic leads. Similar to humans, the worm model of ALS shows protein aggregation, movement defects, reduction of neuron-to-muscle signals, and a shorter lifespan. These ALS-like phenotypes result from the expression of a mutant form of a human disease-causing gene called SOD1. So far, I have successfully identified 4 molecules that extend lifespan of the ALS worms. We hypothesize that these molecules will either reduce SOD1 aggregation, or mitigate its negative effects on the animal’s physiology. In this project, I will continue to screen for molecules that extend lifespan of the worm ALS model, I will test the current and future hits for their ability to suppress the above mentioned ALS phenotypes, and finally test the most promising molecules in mouse models of ALS.

Constanza Cortes Ph.D.

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

DG TFEB-mediated autophagy dysregulation in SBMA

$59,271.00 8/1/2013 7/31/2014 Year 1

$58,491.00 8/1/2014 7/31/2015 Year 2

$59,648.00 8/1/2015 7/31/2016 Year 3

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

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

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

RG The human skeletal muscle cell glycome - structures and functions

$135,000.00 2/1/2013 1/31/2014 Year 1

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

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

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

Carmen Bertoni Ph.D.

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

$100,000.00 8/1/2013 7/31/2014 Year 1

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

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

Duchenne Muscular Dystrophy (DMD) is a genetic disorder caused by the absence of a protein called dystrophin. To date there is no effective cure for DMD and the best option to treat the disease is to restore
expression of dystrophin. Our research group has pioneered the use of gene editing strategies for the dystrophin gene to permanently correct the DNA: the source of the problem. DNA contains the information needed by every cell, including muscles, to function properly. In DMD patients the DNA that makes up the dystrophin gene contains errors. We can use oligonucleotides to let the muscle know of those errors and give the opportunity to the cell that compose each muscle to correct the mistake. We have shown that oligonucleotides can treat mouse models for DMD. In this proposal we intend to compare the efficacy of oligonucleotides to that obtained using a new generation of gene editing tools called Transcription Activator–Like Effecter Nucleases (TALENs) and Transcription Activator–Like Effecter Nickases (TALENickases) and determine whether we can increase the efficiency of the repair to levels suitable to treat the disorder. Comparison will be performed at first in culture using muscle cells isolated from a mouse model for DMD and then in a DMD mouse model to determine the feasibility of using this technology in patients. Each one of these steps is necessary to ensure a safe and effective treatment to human patients.

Rachelle H. Crosbie-Watson Ph.D.

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

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

Bennett Novitch Ph.D.

**RG**  
Developmental mechanisms controlling respiratory motor functions  
$100,000.00 8/1/2013 7/31/2014 Year 1  
$100,000.00 8/1/2014 7/31/2015 Year 2  
$100,000.00 8/1/2015 7/31/2016 Year 3

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

Melissa Spencer Ph.D.

**RG**  
Investigation of osteopontin and inflammatory processes in mdx mice  
$125,000.00 8/1/2013 7/31/2014 Year 3

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

**RG** Mechanisms involved in calpainopathies

$130,000.00 8/1/2013 7/31/2014 Year 2

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

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

Julio Vergara Ph.D.

**RG** Calcium release alterations in malignant hyperthermia and central core disease

$100,000.00 8/1/2013 7/31/2014 Year 1

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

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

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

Oakland - Children's Hospital & Research Center Oakland

Julie Saba M.D.

**RG** Sphingosine-1-phosphate signaling in muscle regeneration and homeostasis

$130,231.00 2/1/2013 1/31/2014 Year 2

$130,231.00 2/1/2014 1/31/2015 Year 3

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

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

Thomas Rando M.D., Ph.D.

**RG** Mechanisms of Fibrosis in Muscular Dystrophies

$125,000.00 8/1/2013 7/31/2014 Year 3
**Summary**

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

**Palo Alto - Stanford University**

**Aaron Gitler Ph.D.**

**RG**

Defining a novel role of profilin 1 in ALS pathogenesis

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

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

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

**Summary**

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

**Andrew Tri Van Ho Ph.D.**

**DG**

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

$60,000.00 2/1/2013 1/31/2014 Year 2

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

**Summary**

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

**Lawrence Steinman M.D.**

**RG**

Immune Tolerance to AAV and Dystrophin for Gene Therapy

$102,687.00 2/1/2013 1/31/2014 Year 2

$102,687.00 2/1/2014 1/31/2015 Year 3

**Summary**

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

**Pasadena - California Institute of Technology**

**David Chan MD/PhD**

**RG**  Mitochondrial dynamics as a protective factor in mitochondrial myopathies  
$84,600.00  5/1/2014  4/30/2015  Year 1  
$84,600.00  5/1/2015  4/30/2016  Year 2  
$84,600.00  5/1/2016  4/30/2017  Year 3

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

**San Diego - San Diego State University Research Foundation**

**Sanford I. Bernstein Ph.D.**

**RG**  Disease mechanism and therapy development for inclusion body myopathy type 3  
$123,437.00  2/1/2013  1/31/2014  Year 2  
$123,437.00  2/1/2014  1/31/2015  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 - California Pacific Medical Center**

**Robert G Miller MD**

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

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

**San Francisco - The Regents of the University of California, San Francisco (Contracts & Grants)**

**Eric Jinsheng Huang M.D., Ph.D.**

**RG**  Mechanisms of FUS mutations in motor neuron survival and synaptogenesis  
$137,500.00  2/1/2013  1/31/2014  Year 2
ALS is caused by the selective degeneration of motor neurons in the central nervous system. Patients with ALS suffer from severe muscle wasting/weakness and eventually die from respiratory failure. Recent genetic data have shown that mutations in the FUS gene can be identified in more than 5% of patients with familial ALS. One important pathological feature in familial ALS with FUS mutations is the presence of abnormal protein aggregates in motor neurons prior to their degeneration. However, it is unclear if abnormal protein aggregates directly contribute to the degeneration of the motor neurons. Based on the function of FUS as a RNA binding protein, we hypothesize that mutation in FUS interferes with normal RNA/protein synthesis, which ultimately leads to cell death and the degeneration of motor neuron synapses. Our goal is to establish both cellular and transgenic mouse models to determine how mutant FUS proteins lead to neuronal cell death and the maintenance of the neuromuscular junction. In support of this view, our data indicate that spinal motor neurons in transgenic mice expressing mutant FUS proteins show severe disruption in RNA and protein synthesis machinery. These exciting results provide strong support that the models we established will provide novel platforms to identify therapeutic targets to block cell death in motor neurons and to restore innervation at the neuromuscular junction.

COLORADO

Aurora - University of Colorado Denver, AMC and DC

Kurt Beam Ph.D.

Analyzing DHPR-RyR1 interactions in a reduced system

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

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

Leslie Leinwand Ph.D.

Mechanisms of Myopathy Caused by Mutations in the Myosin Rod

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

Fort Collins - Colorado State University

Eric Ross Ph.D.

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

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

DISTRIBUTION OF COLUMBIA

Washington - Children's Research Institute (CNMC)

Sebahattin Cirak Ph.D

DG

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

$60,000.00 2/1/2013 1/31/2014 Year 1
$60,000.00 2/1/2014 1/31/2015 Year 2
$60,000.00 2/1/2015 1/31/2016 Year 3

Summary

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

Eric Hoffman Ph.D.

RG

Asynchronous remodeling: A force driving failed regeneration in DMD.

$108,743.00 2/1/2013 1/31/2014 Year 1
$105,339.00 2/1/2014 1/31/2015 Year 2
$107,577.00 2/12/2015 1/31/2016 Year 3

Summary

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

Terence Anthony Partridge Ph.D.

RG

Measuring the dynamics of muscle growth and disease in the mouse model of DMD

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

Summary

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

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

Terence Anthony Partridge Ph.D.

Pre-clinical efficacy testing of Tricyclo antisense oligonucleotides for DMD

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

Washington - Childrens Research Institute

Jyoti Kumar Jaiswal Ph.D.

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

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

Washington - The George Washington University

Maria Chiara Manzini Ph.D.

Unraveling the phenotypic variability of alpha-dystroglycanopathies
**Summary**

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

**FLORIDA**

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

**Ellen Faye Barrett Ph.D.**

**Preserving motor nerve terminals in mouse models of familial ALS**

**RG**

$99,034.00  
2/1/2013 7/31/2014 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.

**Gavriel David Ph.D., M.D.**

**Calcium pathways in peripheral myelinated axons**

**RG**

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

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

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

**Summary**

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

**Carlos T. Moraes Ph.D.**

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

Gainesville - University of Florida

Celine Baligand Ph.D.

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

$57,105.00 2/1/2013 1/31/2014 Year 2

$53,951.00 2/1/2014 1/31/2015 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

$59,925.00 2/1/2013 1/31/2014 Year 2

$59,925.00 2/1/2014 1/31/2015 Year 3

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

Laura P.W. Ranum Ph.D.

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

$138,364.00 2/1/2013 1/31/2014 Year 2

$138,364.00 2/1/2014 1/31/2015 Year 3

Summary We have discovered a new type of translational mechanism in which microsatellite repeat sequences direct the expression of proteins in all three reading frames in the absence of the normal regulatory signals. We call this process repeat associated non-ATG (RAN) translation. We have evidence that this process results in
the expression of unexpected mutant proteins in myotonic dystrophy. Specifically, we have data showing the expression and accumulation of a homopolymeric polyglutamine expansion protein in DM1 patient cells and mice. The goal of this project is to better understand the potential effects of RAN-translation in myotonic dystrophy.

**Maurice Swanson Ph.D.**

**RG** Circadian Clock Dysregulation in Myotonic Dystrophy

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

**Miami - University of Miami School of Medicine**

**Stephan Zuchner M.D.**

**RG** Gene identification in axonal CMT families

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

**GEORGIA**

**Atlanta - Emory University**

**Ayan Banerjee Ph.D.**

**DG** Regulation of PABPN1: Implications for Oculopharyngeal Muscular Dystrophy

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**Summary** The muscle disease called oculopharyngeal muscular dystrophy (OPMD) typically afflicts patients in their 4th or 5th decade of life and causes the most problems with eyelid muscles and muscles required for swallowing. Although we know what gene (the nuclear nuclear poly(A)-binding protein 1, or PABPN1 gene), is altered in this disease, we do not understand why this change causes a muscle disease and we also do not currently have any treatment for this fatal disease. The goal of this proposal is to understand how the protein that is defective in OPMD, PABPN1, is regulated. If we can understand how the function of PABPN1 can be modulated, we may be able to develop new therapeutic approaches to treat OPMD.

**Gary Bassell Ph.D.**

**RG** RNA localization defects in SMA

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

Jonathan D. Glass MD

Clinical, Pathological, and Proteomic correlations in ALS

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

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

A comprehensive approach to identifying novel genes associated with NMDs

A comprehensive approach to identifying the causative gene and understanding the underlying mechanism associated with each disease genotype is inevitable to diagnose disease and eventual selection of effective therapeutic strategy. In this project we will screen a large set of patient samples with known and unknown forms of muscular dystrophies by whole exome sequencing and targeted array analysis to identify the causative gene and the associated genotypes. For each identified candidate gene and genotype, we will later perform confirmation studies by transcript expression analysis (qRT-PCR) and western blot analysis to understand the nature of substantial alterations in the expression patterns of the muscle proteins. Mutations in a single gene and altered levels of the corresponding protein may further alter the expression pattern of closely related proteins, especially in the case of muscle proteome where several proteins form structural complexes, thereby modifying in these brains (proteomics) looking for patterns or “signatures” of disease that can differentiate ALS patients with and without dementia. Finally, we will test each patient for genetic mutations that may occur ALS and FTD as a further differentiating factor.

Grace Pavlath Ph.D.

A KnockIn Mouse Model for Oculopharyngeal Muscular Dystrophy

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

Augusta - Georgia Regents University

Lin Mei M.D./Ph.D.

Mechanisms of LRP4 autoantibodies in myasthenia gravis

This study aims to understand the role of LRP4 autoantibodies in myasthenia gravis (MG), a disease characterized by muscle weakness due to the presence of these antibodies. By elucidating the mechanisms by which LRP4 is targeted in MG, this research could provide new therapeutic strategies for this debilitating condition.
Myasthenia gravis (MG) is caused by autoantibodies against muscle nicotinic acetylcholine receptor (AChR) and MuSK, a receptor tyrosine kinase that is critical for agrin-induced AChR concentration at the neuromuscular junction (NMJ). However, some MG patients are negative for autoantibodies against AChR or MuSK. A better understanding of the pathogenic mechanisms of "seronegative" MG should have a major impact on diagnosis and treatment of these patients. In preliminary studies we found that sera of "seronegative" patients contained autoantibodies against LRP4, a receptor of agrin essential for NMJ formation. This result is exciting, but raises a critical question whether the LRP4 autoantibodies are pathogenic and if so, what the underlying mechanisms are. We will address these questions in this proposal. Results of the proposed research should contribute a better understanding of "seronegative" MG and development of novel diagnostic and therapeutic strategies for this devastating disease.

ILLINOIS

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

Steven C. Zimmerman Ph.D.

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

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

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

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

Christine DiDonato PhD

RG SMN inductive therapy in mild SMA

$135,000.00 2/1/2013 1/31/2014 Year 1
$135,000.00 2/1/2014 1/31/2015 Year 2
$135,000.00 2/1/2015 1/31/2016 Year 3

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

Chicago - Illinois Institute of Technology

Nick Menhart Ph.D.

RG Biophysics of Exon Skipped Dystrophin Rods

$85,837.00 2/1/2013 1/31/2014 Year 2
$85,837.00 2/1/2014 1/31/2015 Year 3

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

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

Muthusamy Thiruppathi Ph.D

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

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

Chicago - The University of Chicago

JianRong Sheng Ph.D

RG Immunomodulation of Experimental Autoimmune Myasthenia Gravis

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

Hines - Chicago Association for Research & Education in Science

Junping Xin Ph.D.

DG Characterization of CD4+ T cell response in ALS mouse following nerve injury

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.

INDIANA

Indianapolis - Indiana University (Indianapolis)

Ronald Mark Payne M.D.

RG  Mechanism of Heart Failure in Friedreich Ataxia
$149,024.00  2/1/2013  1/31/2014  Year 1
$149,024.00  2/1/2014  1/31/2015  Year 2

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

West Lafayette - Purdue University

Shihuan Kuang Ph. D.

RG  Targeting hypoxia signaling to improve the efficiency of myoblast transfer
$84,600.00  5/1/2014  4/30/2015  Year 1
$84,600.00  5/1/2015  4/30/2016  Year 2
$84,600.00  5/1/2016  4/30/2017  Year 3

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

IOWA

Iowa City - The University of Iowa

Kevin Peter Campbell PhD

RG  Protein O-mannosylation: Classification of new players in muscular dystrophy
$125,000.00  8/1/2013  7/31/2014  Year 2
$125,000.00  8/1/2014  7/31/2015  Year 3

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

Jennifer Rachel Levy Ph.D.

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

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

Michael Shy M.D.

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

$145,917.00 1/1/2014 12/31/2014 Year 2
$147,951.00 1/1/2015 12/31/2015 Year 3

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

Michael Shy M.D.

RG Identification and Treatment of ER Stress in Patients with CMT1

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

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

MARYLAND

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

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

RG Myostatin Regulates Fate of Satellite Cells in Dystrophic Muscle

$117,513.00 8/1/2013 7/31/2014 Year 3

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

Baltimore - Johns Hopkins University School of Medicine

Elizabeth H. Chen Ph.D.

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

$107,163.00 2/1/2013 1/31/2014 Year 2
$107,163.00 2/1/2014 1/31/2015 Year 3

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

**Mohamed H. Farah PhD**

**RG** Enhancing neuromuscular reinnervation by BACE1 inhibition

$125,000.00 2/1/2013 1/31/2014 Year 1

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

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

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

**Youngjin Lee Ph.D.**

**DG** Glial monocarboxylate transporters (MCTs) pathway in neurodegeneration

$59,999.00 8/1/2013 7/31/2014 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.

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

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

$130,902.00 2/1/2013 1/31/2014 Year 1

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

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

**Summary** Understanding the pathophysiology and development of new therapeutics for amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig’s Disease) and dementias such as Alzheimer’s and frontotemporal dementia has been an enormous challenge. The ability to actually have human cell lines that represent the natural disease by carrying hereditary gene mutations will provide unprecedented tools. In this application we propose to study molecular events that may contribute to the disease of a newly discovered common gene mutation in ALS (C9ORF72) which is found in inherited (familial) as well as the common sporadic forms of ALS. We will employ ALS patient-derived human fibroblasts and convert them into adult induced pluripotent stem (iPS) cells as well as differentiated relevant central nervous system (CNS) cell types such as astroglia and motor neurons. These human cells will undergo a thorough analysis of their molecular genetic composition, which will then be compared to the genetic profile of human cells obtained from normal, healthy volunteers. Based on the differences we will design and develop a molecular therapeutic agent targeted at the specific mutation responsible for the disease. We will further develop a so called biomarker which will allow us to non-invasively monitor the efficacy of these novel drugs when given to patients. The use of these human cells may allow us to efficiently and quickly develop a drug therapy for C9ORF72 form of
ALS.

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

RRG Robert Packard Center for ALS Research (Wings 2012) (Rothstein, Jeffrey)

$121,376.18 8/1/2013 7/31/2014 Year 1

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

Charlotte Jane Sumner M.D.

RG Characterization of TRPV4 associated peripheral neuropathy in animal models

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

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

Baltimore - Johns Hopkins University-School of Medicine

Amanda M. Haidet-Phillips Ph.D

DG Investigation into astrocyte-mediated degeneration of upper motor neurons in ALS

$50,239.00 5/1/2014 4/30/2015 Year 1
$50,239.00 5/1/2015 4/30/2016 Year 2
$50,239.00 5/1/2016 4/30/2017 Year 3

Summary Amyotrophic Lateral Sclerosis (ALS) results in the impairment of upper motor neurons (MNs) in the motor cortex and lower alpha MNs in the spinal cord. A fundamental shortcoming of the ALS community’s research is the relatively small number of basic science and clinical investigations into upper MN dysfunction in ALS patients. This discrepancy is due to several factors including difficulty in objectively assessing upper MN findings clinically as well as the lack of cellular and molecular tools and animal models available to study upper MNs. However, the discovery of novel markers to identify upper MNs now allows for these investigations. One of the major disease pathways influencing lower MN death in ALS is through non-neuronal glial cells such as astrocytes. Recently, astrocytes derived from familial and sporadic post-mortem ALS patients were shown to cause the death of lower MNs in culture. In this proposal, we will investigate whether upper MNs are also pathologically influenced by astrocytes. We will use astrocytes taken from ALS mouse models as well as astrocytes derived from stem cells created from ALS patients. The results may begin to offer critical insight into whether upper MNs degenerate through similar or divergent mechanisms as lower MNs in ALS. Further understanding of these mechanisms can guide the development of therapies targeting upper MNs specifically or therapies which may be beneficial for upper and lower MNs.

Baltimore - University of Maryland, Baltimore

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

RG Cellular and Molecular Studies of Dysferlinopathy

$120,765.00 2/1/2013 1/31/2014 Year 2
$120,765.00 2/1/2014 1/31/2015 Year 3

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

**MASSACHUSETTS**

**Boston - Brigham and Women's Hospital, Inc.**

**Xin Wang Ph.D.**

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

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

**Boston - Children’s Hospital Boston**

**Matthew Alexander Ph.D.**

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

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

**Alan H. Beggs Ph.D.**

**RG**  Molecular Genetics of Congenital Myopathies

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

**Emanuela Gussoni Ph.D**

**RG**

Melanoma cell adhesion molecule (MCAM) in human myogenic cells

$128,022.00 8/1/2013 7/31/2014 Year 3

**Summary**

The direct injection of normal cells into mice and patients with muscular dystrophy has been attempted as a way to provide missing proteins, such as dystrophin, to the affected muscles. These studies have shown that, while the technique is safe, the muscles of patients do not show significant improvement. One of the causes for this inefficiency is the rapid death of the normal cells following injection. To overcome this problem, different cell types more capable of surviving the transplant and producing the desired protein must be identified. We will study new sub-fractions of human cells isolated based on the expression of the surface protein melanoma cell adhesion molecule (MCAM). We have preliminary evidence that MCAM-expressing cells are capable of forming muscle both in tissue culture and following injection into animals. We seek to determine: 1) whether cells expressing this protein are pre-destined to become muscle, 2) how expression of MCAM protein is linked to myogenic potential and 3) whether fractions of human cells expressing MCAM can efficiently repair dystrophic muscle. These studies will help elucidate the function of MCAM in human muscle cells and determine whether MCAM-expressing cells are promising candidates for translational studies.

**Jianming Liu Ph.D**

**DG**

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

$59,997.00 2/1/2013 4/30/2014 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.

**Fedik Rahimov Ph.D.**

**DG**

Biomarker Discovery in Muscles from FSHD Patients

$60,000.00 8/1/2013 7/31/2014 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.

**Da-Zhi Wang Ph.D.**

**RG**

The miR-155-MEF2A axis in muscular dystrophy

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

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

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

**Summary**

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

Boston - Dana-Farber Cancer Institute
Pere Puigserver PhD
RG Role of mTOR/YY1/PGC1 transcriptional complex in mitochondrial myopathies.
$101,443.00 8/1/2013 7/31/2014 Year 3
Summary Mitochondria are the energetic power plants of the cell that use nutrients as a fuel to obtain energy required to maintain cellular functions and survival. Failure to activate normal mitochondrial function is a key feature of mitochondrial myopathies, a group of neuromuscular disorders, often caused by mutations affecting proteins from this organelle. There is currently no therapy to treat mitochondrial myopathies which present a progressively severe neuromuscular dysfunction. The transcriptional metabolic coactivator PGC-1a rescues some of the defective mitochondrial function both in cell culture and mouse models of mitochondrial myopathies. Importantly, activation of PGC-1a function prevented a energetic deficit and effectively improve the mitochondrial myopathy phenotype in mouse models. Thus, activation of PGC-1a, or components of its molecular pathway, could be a treatment for mitochondrial myopathy patients. However, because PGC-1a is a transcriptional coactivator small molecules that bind directly and activate its function are unlikely to be found. Based on our knowledge of how the PGC-1a pathway is activated, here we propose two new strategies. First, activation of PGC-1a through the mTOR/YY1 complex and, second, small molecule chemical screening to identify compounds that activate PGC-1a using cybrid cell lines carrying genetic mutations from mitochondrial myopathy patients.

Boston - Harvard Medical School
Alfred L. Goldberg Ph.D.
RG Protein breakdown in muscle in normal and disease states
$135,399.00 8/1/2013 7/31/2014 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.

Edward Owusu-Ansah Ph.D
DG A Molecular Genetic Analysis of Mitochondrial Myopathy
$60,000.00 8/1/2013 7/31/2014 Year 3
Summary A functional oxidative phosphorylation (OXPHOS) system is crucial for the generation of ATP (energy) in mitochondria. Accordingly, disruption of this system can compromise a range of biochemical and metabolic activities in cells, resulting in several muscle and neurodegenerative diseases. Mitochondrial Complex I deficiency is associated with many mitochondrial diseases, yet the molecular pathways that are disrupted upon complex I disruption, which trigger downstream signaling events associated with disease, remain largely unresolved. Damaged mitochondria compensate by increasing the expression of genes that allow survival under suboptimal conditions. Most of these damage-induced genes are involved in either repairing the damaged mitochondria or making new mitochondria. The full complement of cellular processes activated to restore or maintain viability in the presence of damaged mitochondria constitute an adaptive cytoprotective response. We have established a paradigm in the model organism Drosophila to study...
cytoprotective factors activated in response to mitochondrial perturbation. Due to the extensive similarity between Drosophila and human genomes, we anticipate that information obtained from this study should uncover novel therapeutic strategies for alleviating mitochondrial diseases in humans, and ultimately the aging process.

**Boston - Harvard University School of Public Health**

Marc Weisskopf Ph.D., Sc.D.

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

$100,538.00 8/1/2013 7/31/2014 Year 2

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

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

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

Vera Fridman M.D.

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

$90,000.00 9/16/2013 9/15/2014 Year 1

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

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

Thurman Wheeler M.D.

**RG** Progressive myopathy and therapeutic development in myotonic dystrophy type 1

$132,000.00 8/1/2013 7/31/2014 Year 2

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

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

**Boston - Trustees of Boston University**

Mahasweta Girgenrath Ph.D

**RG** Modulation of Inflammation in the context of Regeneration in MDC1A
**Summary**

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

**Jeffrey Boone Miller PhD**

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

$114,620.00 2/1/2013 1/31/2014 Year 2
$114,620.00 2/1/2014 1/31/2015 Year 3

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

**Concord - Valerion Therapeutics, Inc.**

**Dustin Armstrong Ph.D.**

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

$393,311.00 1/1/2014 7/31/2014 Year 2
$661,839.00 8/1/2014 2/28/2015 Year 3

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

**Worcester - University of Massachusetts Medical School**

**Charles P. Emerson Ph.D.**

**RG**
Evaluating DUX4 as a therapeutic target for FSHD

$176,470.00 2/1/2013 1/31/2014 Year 2
$176,470.00 2/1/2014 1/31/2015 Year 3

**Summary**
Facioscapulohumeral muscular dystrophy (FHS4) is linked to abnormalities on a section of chromosome 4 (4q35). These genetic and/or epigenetic abnormalities appear to lead to aberrant expression of a potentially toxic protein, named DUX4-fl, and this mis-expression of DUX4-fl is currently thought to underlie the development of muscle weakness in FSHD. However, many questions remain about how DUX4-fl mis-expression occurs in diseased muscle and how therapies might be designed to inhibit pathology caused by DUX4-fl. Our studies therefore are designed to answer important questions about how DUX4-fl expression is regulated. In particular, we will investigate the connection between changes in the DUX4 gene and expression of the toxic form of this gene to identify new therapeutic targets for FSHD. In addition, we will identify and validate potential targets for small molecule therapies. Furthermore, we will determine if drugs affecting the regulation of the DUX4 locus can eliminate expression of the toxic protein, raising their potential as FSHD therapeutics. We expect that the results of our studies will provide critical information needed to develop effective and specific DUX4-targeted therapies for FSHD.

**Rossella Tupler M.D., Ph.D.**

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

MICHIGAN

Ann Arbor - Nymirum, Inc.

Michael Pape PhD

RG SAR for Small Molecules Targeting Toxic CUG Expanded Repeats.

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

Ann Arbor - The Regents of the University of Michigan

Anthony Antonellis Ph.D.

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

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

Andrew Lieberman M.D., Ph.D.

RG Allosteric activators of Hsp70 to treat spinobulbar muscular atrophy

Summary
Spinobulbar muscular atrophy (SBMA) is an inherited degenerative disorder of lower motor neurons that is caused by a CAG/glutamine tract expansion in the androgen receptor (AR) gene. The mutant protein causes testosterone-dependent toxicity that results in muscle weakness and atrophy in men. Prior work has established that the mutant AR protein is the cause of this toxicity, suggesting that strategies to enhance its degradation should diminish disease severity. Therefore, we sought to understand this process, and found that AR degradation is tightly controlled by a cellular machinery consisting of the heat shock protein 70 (Hsp70). We propose that stabilizing Hsp70 in a conformation that binds the mutant AR with high affinity will promote its degradation. We will test this idea both genetically, using an Hsp70 interacting protein that stabilizes Hsp70 in its high affinity binding state, and pharmacologically, using a novel small molecule that we recently identified which functions similarly. We will also test a small molecule that activates Hsp70's binding to the mutant AR in SBMA mice. We hypothesize that promoting Hsp70 binding to the mutant AR will increase its degradation and alleviate toxicity in SBMA models. It is our expectation that this work will help define a new therapeutic approach to SBMA and other protein aggregation disorders where degradation of the mutant protein is controlled by Hsp70.

Daniel E Michele Ph.D.

Reversing nitric oxide synthase dysfunction in muscular dystrophy

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

MINNESOTA

Minneapolis - Regents of the University of Minnesota - Twin Cities

Atsushi Asakura Ph.D.

Angiogenesis-based therapy for muscular dystrophy

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

James M. Ervasti Ph.D.

Biophysical Optimization of Therapeutic Dystrophin Constructs

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

**Michael Kyba PhD**

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

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

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

**Joseph M Metzger Ph.D.**

**Development and testing of membrane sealants for muscular dystrophy**

$118,934.00 8/1/2013 7/31/2014 Year 2

$.00 8/1/2014 7/31/2015 Year 3

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

**Rita R. Perlingeiro Ph.D.**

**DMD IPS CELLS: GENETIC CORRECTION AND MUSCLE REGENERATION**

$130,000.00 8/1/2013 7/31/2014 Year 2

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

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

**David D. Thomas Ph.D.**

**Muscular dystrophy therapy based on small-molecule activators of Ca2+ transport**

$130,000.00 2/1/2013 1/31/2014 Year 2

$130,000.00 2/1/2014 1/31/2015 Year 3

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

**Rochester - Mayo Clinic.**

**Andrew George Engel M.D.**

**Restricted funds for congenital myasthenia gravis research**

1/1/2014 12/31/2015 Year 3
**Summary**

**MISSOURI**

**Columbia - The Curators of the University of Missouri**

*Dongsheng Duan PhD*

**RIG**  
A canine tissue bank for Duchenne muscular dystrophy study  
$84,600.00  5/1/2014  4/30/2015  Year 1  
$84,600.00  5/1/2015  4/30/2016  Year 2

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

*Christian Lorson PhD*

**RG**  
Evaluation of SMA pathways with scAAV9 vectors  
$127,194.00  2/1/2013  1/31/2014  Year 2  
$127,194.00  2/1/2014  1/31/2015  Year 3

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

**St. Louis - Washington University in St. Louis**

*Elisabetta Babetto Ph.D.*

**DG**  
Phr1 as a novel regulator of axon integrity in Charcot-Marie-Tooth diseases  
$50,760.00  5/1/2014  4/30/2015  Year 1  
$50,760.00  5/1/2015  4/30/2016  Year 2  
$50,760.00  5/1/2016  4/30/2017  Year 3

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

*Bogdan Karl Beirovski M.D., Ph.D.*

**DG**  
Modeling axon loss in CMT by disruption of Schwann cell metabolic regulation  
$60,000.00  8/1/2013  7/31/2014  Year 2  
$60,000.00  8/1/2014  7/31/2015  Year 3

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

Martha Bhattacharya Ph.D.

**DG** Molecular Mechanisms of Peripheral Axonal Degeneration

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

Anne M Connolly M.D.

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

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

Jeffrey D. Milbrandt MD, PhD

**RG** Manipulating Schwann cell metabolism to treat peripheral neuropathy

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

**Timothy M Miller M.D., Ph.D.**

**HCTG**  
Natural History Study of Familial ALS

$59,500.00  
1/1/2014  
12/31/2014  
Year 3

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

**Kelly Renee Monk Ph.D.**

**RG**  
Control of myelination by G protein-coupled receptor signaling

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

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

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

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

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

**RG**  
Autophagic treatment paradigms for desminopathies

$132,074.00  
2/1/2013  
1/31/2014  
Year 2

$132,074.00  
2/1/2014  
1/31/2015  
Year 3

**Summary**  
Protein aggregate myopathies are a group of devastating muscular disorders with common pathologic features. These include focal disruption of myofibrils, the accumulation of undegraded myofibrillar proteins and the ectopic aggregation of multiple proteins including desmin, amyloid precursor protein (APP), TAR DNA-binding protein 43 (TDP-43), âB-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 âB-crystallin knockin mouse model. This mouse expresses mutant R120G âB-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 âB-crystallin, desmin and ubiquitin. We have developed assays to measure the autophagy in skeletal muscle. We will use these assays to define FDA approved drugs with reported autophagic activity. These drugs will then be used to treat an animal model of desmin related myopathy. Upon completion of these studies we will know if autophagic enhancement is a viable therapeutic approach in this devastating disorder.

**NEVADA**

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

**Dean J. Burkin Ph.D.**

**RG**  
Laminin-111 protein therapy for Duchenne Muscular Dystrophy

$102,676.00  
8/1/2013  
7/31/2014  
Year 2

$102,676.00  
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Year 3

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

**Ryan David Wuebbles Ph.D.**

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

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**Summary** Although there is currently no effective treatment or cure for DMD, several promising therapeutics are currently being investigated. One of these is an extracellular matrix protein called Laminin-111, the embryonic parologue to Laminin-211, which both interact with the dystrophin-associated glycoprotein complex (DGC) and alpha7beta1 Integrin. Within the mdx mouse model of DMD, the introduction of Laminin-111 leads to the prevention of muscle pathology and reduced exercise-induced muscle injury. These changes were likely brought about through the increased levels of both Utrophin and alpha7 Integrin proteins. However, the production of laminin-111 protein for the use as a therapy for DMD is difficult due to the size of the heterotrimeric protein of over 900 kDa. These exciting results will be more quickly and easily brought into therapeutic use if a smaller part of the Laminin-alpha1 protein or peptide is capable of reproducing the effects of the entire laminin-111 protein complex. Here, we propose to determine if part of the Laminin-alpha1 protein is capable of producing the therapeutic effects of the entire complex. The results of this study could provide a novel protein therapy for DMD which would be more quickly and easily produced than that of the entire complex.

**NEW JERSEY**

**Bridgewater - SANOFI-AVENTIS U.S. INC**

**Christopher Penton N/A**

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

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

**Newark - Rutgers, The State University -Newark Campus**

**Diego Fraidenraich Ph.D.**

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

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

**NEW MEXICO**

**Albuquerque - University of New Mexico HSC**

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

**NEW YORK**

**Albany - Research Foundation of SUNY - University at Albany**

Li Niu Ph.D.

**Characterization of Chemically Modified Aptamers as New ALS Drug Candidates**

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**Summary**

Excessive activation of the a-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtype of ionotropic glutamate receptors is an important pathogenic mechanism for ALS. Finding inhibitors to control the excessive receptor activity has been a long-pursued strategy for developing ALS drugs. We previously showed that nanomolar affinity RNA inhibitors or RNA aptamers selectively targeting AMPA receptors can be identified. These aptamers are superior to traditional, small-molecule inhibitors, because these traditional inhibitors are organic compounds and generally have poor water solubility, low affinity and cross activity. However, unmodified, these RNA aptamers are limited in therapeutic applications in vivo by their inherent sensitivity towards ribonucleases, the enzymes that catalyze RNA degradation. In contrast, chemical modifications of RNA molecules can turn them into ribonuclease-resistant or biostable aptamers. Thus, making biostable aptamers is the first step to translate these powerful AMPA receptor aptamers into clinically useful drugs. Thus far, we have successfully developed several high-affinity, chemically modified aptamers for AMPA receptors. The goal of this proposal is to characterize these chemically modified RNA aptamers for their neuroprotective effectiveness on glutamate-induced neurotoxicity in ALS cellular and animal models. These studies are key preclinical experiments to advance these RNA inhibitors as a new ALS drug.

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

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

**Mechanisms of CNS Disease in X-Linked CMT**

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**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.

**Ithaca - Cornell University**

Fenghua Hu Ph.D.
Role of Ubiquitination in TDP-43 aggregation and clearance

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

New York - Columbia University Medical Center

Tomoyuki Awano Ph.D.

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

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.

Veronica Hinton Ph.D.

Executive Functions in Boys with Dystrophinopathy

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

Michio Hirano M.D.

Molecular bypass therapy for TK2 deficiency
Thymidine kinase 2 (TK2) deficiency is a rare genetic neuromuscular disease that typically begins in infancy and is fatal in childhood but can also manifest as adult-onset progressive external ophthalmoplegia. We have generated a mouse model with severely decreased Tk2 activity and reductions in its products. In preliminary studies, administration of compounds to bypass the defective Tk2 enzyme slowed the progression of the disease and extended the lifespans of the mutant mice. Moreover, we demonstrated that the compound is able to penetrate into tissues including the brain. We propose to characterize the cause of the neuromuscular weakness and to optimize long-term treatment in the mutant mice. If we are successful, our studies may lead to a significant therapy for human TK2 deficiency and related diseases.

**Ronald K. Liem Ph.D.**

**Characterization of a new mouse model for CMT2E**

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

**Hiroshi Mitsumoto MD**

**2011-2012 Wings Four Independent Proposals**

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We propose 4 independent projects. Project 1 aims to investigate whether or not oxidative stress is increased in ALS patients with impending respiratory failure. With successful treatment with non-invasive ventilators (NIV), oxidative stress may be significantly reduced along with an increased quality of life. For project 2, we will study metabolic markers, mitochondrial dysfunction, and oxidative stress in fibroblasts from ALS patients, in collaboration with Dr. Giovanni Manfredi, Weill Cornell Medical School. We will attempt to discover if ells outside of the nervous system would provide a clue for the pathogeneses of the disease. In project 3, we will prepare a new study to prospectively look at the natural history of pure upper motor neuron dysfunction/disease (PUMND) and early PLS. Project 4 aims to explore the feasibility of studying epigenetic in ALS. We are collecting RNA, skin fibroblast and autopsy tissue to identify the best way to study epigenetic changes in ALS. We will prepare a future large project in epigenetic studies, which is novel but requires a fundamental exploration for the methodologies and effective objectives. The MDA Wings Over Wall Street Funds have been a driving force for our innovative and highly active research projects at the Eleanor and Lou Gehrig MDA/ALS Research Center.

**Hiroshi Mitsumoto MD**

**2012 Wings Over Wall Street Research Projects Proposal**

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

**Hiroshi Mitsumoto MD**

**2013 Wings Over Wall Street Research Projects Proposal**

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

Umrao R. Monani Ph.D.

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

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

Catarina M. Quinzii M.D.

Investigating the Pathogenesis of Encephalomeiomyopathy due to RMND1 mutations

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Mitochondria are often described as the “powerhouses of the cell” because these tiny structures generate most of the body’s energy by converting carbohydrates, fats, and proteins to water and carbon dioxide. Mitochondria are unique constituents of human cells because they are the products of two types of genetic material: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Defects of either nDNA or mtDNA can cause mitochondrial dysfunction, which frequently affects brain and muscle, which require abundant energy. Disorders of mitochondrial protein synthesis have been reported in a heterogeneous group of patients, mostly presenting with early-onset, lethal diseases. Recently, in a patient with a new fatal, early-onset encephalomyopathy and impaired mitochondrial protein translation, we identified a mutation in the gene encoding the required for nuclear meiotic division 1 (RMND1) protein, never before associated with a human disease. We will investigate why abnormal RMND1 causes mitochondrial dysfunction, by studying human RMND1 mutant and RMND1-depleted cells, murine RMND1-depleted embryonic stem cells, and a mouse model. Ultimately, we hope to develop a treatment for this devastating disease.

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

Marilena D'Aurelio Ph.D.

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

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

Giovanni Manfredi M.D., Ph.D.

Summary

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

New York - Memorial Sloan-Kettering Cancer Center

Mary Baylies Ph.D.

Summary

Emery Dreifuss Muscular Dystrophy (EDMD) has been linked to mutations in LMNA, a gene which encodes the Lamin A and C proteins. Lamin A and C are components of the nuclear lamina, a fibrous structure associated with the inner nuclear membrane via interactions with integral membrane proteins. Lamin A and C provide structural integrity and shape to the nucleus. They also interact with chromatin and transcriptional regulators to influence gene expression in myofibers and satellite cells. Recently, EDMD-linked mutations in Lamin A/C also have been shown to cause nuclear movement/positioning defects in tissue culture. Given the many functions of Lamin A/C, the reason why LMNA mutations cause muscle disease remains unclear. We previously identified a microtubule-associated protein, Ensconsin (Ens) as critical for nuclear movement in both Drosophila and mouse muscle. ens mutant larvae do not move as fast as wild-type larvae, indicating that improper nuclear localization has significant impact on muscle function. We find that Ens physically and genetically interacts with Lamin C. Lamin C mutants have mispositioned nuclei and defective muscle function. We hypothesize that Ens and Lamin C act together, linking nuclear positioning to gene expression and muscle function. We will determine the nature of the interaction, how they regulate muscle function, and provide new insights to both the cellular processes required for optimal muscle function and to different muscle diseases.

Sonja Nowotschin Ph.D

Summary

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

**New York - The Hospital for Special Surgery**

**Dale Lange M.D.**

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

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

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

**Eric A. Schon Ph.D.**

**RG** Treatment strategies for human mitochondrial disease

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

**Howard J. Worman M.D.**

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

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

**Rochester - University of Rochester**
Robert Dirksen Ph.D.

RG  Orai1 as a Therapeutic Target for Central Core Disease

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

Summary There are no drug therapies available to treat central core disease (CCD) or environmental heat stress (EHS). The overall objective of this proposal is to evaluate the effectiveness of inhibiting calcium influx through store-operated calcium entry (SOCE) channels as a viable therapy to treat CCD and EHS. We will test the hypothesis that both the mitochondrial myopathy in CCD and excessive heat generation during EHS require uncontrolled calcium influx through SOCE channels. We will determine the effects of innovative mouse genetic and drug interventions to inhibit SOCE on the mitochondrial damage, core myopathy, and heat sensitivity of an established mouse model of EHS with central cores. Effects of SOCE inhibition on the heat stress response of normal mice will also be determined in order to assess the utility of SOCE channel inhibitors in preventing EHS and heat-related illness in normal individuals. In addition to CCD and EHS, alterations in calcium homeostasis and mitochondrial function contribute to multiple other MDA-sponsored muscular dystrophies including Duchenne Muscular Dystrophy, Centronuclear Myopathy, Amyotrophic Lateral Sclerosis, Mitochondrial Myopathy, Myotubular Myopathy, Bethlem Myopathy, and Ullrich Congenital Muscular Dystrophy. Thus, the fundamental discoveries and therapeutic advances accomplished during this project will have broad implications for multiple MDA-supported muscle disorders.

Robert Griggs M.D.

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

$42,840.00  10/1/2013  9/30/2014  Year 1
$64,260.00  10/1/2014  9/30/2015  Year 2
$85,680.00  10/1/2015  9/30/2016  Year 3

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

Jeffrey Statland M.D.

CRTG  Clinical Trial Preparedness in Facioscapulohumeral Dystrophy

$83,709.00  7/1/2013  6/30/2014  Year 2

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

Charles Thornton MD
**Models for therapeutic development in DM1**

$107,271.00 8/1/2013 7/31/2014 Year 2
$94,393.00 8/1/2014 7/31/2015 Year 3

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

**Charles Thornton MD**

**Myotonic Dystrophy Clinical Research Network**

$306,000.00 12/1/2013 11/30/2014 Year 2
$306,000.00 12/1/2014 11/30/2015 Year 3

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

**Syracuse - SUNY Upstate Medical University**

**Jeremy Shefner MD, PhD**

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

$250,000.00 1/1/2014 12/31/2014 Year 3
$125,000.00 1/1/2015 3/1/2015 Year 4

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

**NORTH CAROLINA**

**Chapel Hill - The University of North Carolina at Chapel Hill**

**Joan M. Taylor Ph.D.**

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

$132,000.00 2/1/2013 1/31/2014 Year 1
$132,000.00 2/1/2014 1/31/2015 Year 2
$132,000.00 2/15/2015 1/31/2016 Year 3

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

**Charlotte - Carolinas Medical Center**

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

**RRG** Longitudinal Assessment, Gait Analysis, and Biorepository of LGMD

$222,769.69 10/1/2013 9/30/2014 Year 1

**Summary** Limb-girdle muscular dystrophy (LGMD) is largely a descriptive term for a molecularly heterogeneous group of muscular dystrophies with onset in childhood or adulthood that is characterized by progressive muscle
weakness. LGMD are classified into two groups based on the mode of inheritance, type 1 for autosomal dominant and type 2 for autosomal recessive. Each type is further subdivided depending on the molecular etiology, designated by a letter in the order they were discovered (i.e. LGMD1A-E and LGMD2A-N). Molecular clarification has resulted in the elucidation of common pathways of pathogenesis, as well as important differences between subtypes of LGMD. The community has identified lack of natural history studies as a major gap in our knowledge base and a significant barrier to the development of effective clinical trials in LGMD. In addition, the lack of validated clinical trial endpoints makes it near impossible to transition potential therapeutics through rigorous clinical trials into routine treatments for LGMD. This proposal aims to comprehensively evaluate individuals with genetically identified LGMD and follow potential outcome measures longitudinally in patients with LGMD. In addition, there is an aim to perform gait analysis and collect samples for a LGMD bio-repository.

Durham - Duke University
Charles Alan Gersbach Ph.D.

**Genetic Correction of Duchenne Muscular Dystrophy with Engineered Nucleases**

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

Durham - Duke University Medical Center
Dwight Koeberl M.D., Ph.D.

**Enhanced muscle-targeted gene therapy for Pompe disease**

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

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

**Structural studies of myostatin inhibitors**

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

**Cleveland - Cleveland Clinic Foundation**

**Feng Lin Ph.D.**

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

$130,000.00 8/1/2013 7/31/2014 Year 2

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

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

**Columbus - Research Institute at Nationwide Children’s Hospital**

**Scott Q. Harper Ph.D.**

**RG** Development of An Inducible FSHD Mouse Model

$100,713.00 2/1/2013 1/31/2014 Year 2

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

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

**Columbus - The Ohio State University**

**Noah Weisleder Ph.D.**

**RG** Protein therapy targeting limb girdle muscular dystrophy

$135,000.00 2/1/2013 1/31/2014 Year 1

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

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

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

**Columbus - The Research Institute at Nationwide Children’s Hospital**

**Paul Martin Ph.D.**

**RG** Protein-based GALGT2 therapies for Duchenne muscular dystrophy

$132,000.00 8/1/2013 7/31/2014 Year 3
Summary

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

Jerry Mendell M.D.

CRNG MDA Clinical Network

$306,000.00 11/1/2013 10/30/2014 Year 2
$306,000.00 11/1/2014 10/30/2015 Year 3

Summary

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

Federica Montanaro Ph.D.

RG Defining the role of impaired Hedgehog signaling in DMD

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

Summary

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

Toledo - The University of Toledo

Kenneth Hensley Ph.D.

RG Targeting CRMP2 to Treat Motor Neuron Disease

$108,868.00 2/1/2013 1/31/2014 Year 2
$108,606.00 2/1/2014 1/31/2015 Year 3

Summary

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

OREGON

Eugene - University of Oregon

Andrew Berglund Ph.D.

RG

Stabilization of toxic RNA provides novel insights into myotonic dystrophy

Summary

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

Portland - Oregon Health & Science University

Paul Brehm Ph.D.

RG

Use-dependent fatigue in muscle rapsyn myasthenic syndrome is presynaptic

Summary

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

Michael W. Linhoff Ph.D.

DG

Presynaptic structure and molecular composition in a zebrafish myasthenia model

Summary

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

PENNSYLVANIA

Philadelphia - Philadelphia Health and Education corporation d/b/a Drexel University College of Medicine
Terry Heiman-Patterson MD

RG Identification of modifying genes in murine models of ALS

$110,000.00 2/1/2013 1/31/2014 Year 2

$110,000.00 2/1/2014 1/31/2015 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 - The Children's Hospital of Philadelphia

Masahiro Iwamoto Ph.D.

RG Intervention of muscular dystrophy by selective RARgamma agonist

$135,000.00 2/1/2013 1/31/2014 Year 1

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

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

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

Philadelphia - The Trustees of the University of Pennsylvania

Tathagata Chaudhuri Ph.D.

DG Matrix Conditioning of Mesenchymal Stem Cells to Rescue Muscular Dystrophies

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

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

Summary The specific goal of this study is to develop a novel technology which will direct human Mesenchymal Stem Cells (MSCs) to form muscle cells and therefore repair damaged skeletal muscle in muscular dystrophies, particularly, Duchenne Muscular Dystrophy (DMD). MSCs are commercially available and can be engineered to differentiate into muscle and our objective is to show that matrix based-conditioning of these MSCs can induce differentiation into muscle like cells. First, we will test if these preconditioned MSCs are capable of rescuing muscle defects and lead to myogenesis when injected into the damaged muscles of mdx mice, the mouse model of DMD. Secondly, to examine if this work can be clinically translated, we will also apply the same technology in the golden retriever muscular dystrophy (GRMD) dogs which exhibit a more severe dystrophic phenotype and closely resembles the human condition. We will determine if dog MSCs derived from GRMD dogs can also be programmed by matrix specification into a myogenic fate, similar to human MSCs. Finally, we will engineer these dog MSCs to express dystrophin, commit them into a myogenic lineage and inject them back into the same donor GRMD dog to examine if muscle repair and regeneration occur. These goals will determine if matrix elasticity alone can induce both human and canine MSCs to be committed into a myogenic lineage and whether this approach of utilizing preconditioned cells can be used for cellular therapy of muscular dystrophies.
Tejvir S. Khurana MD, Ph.D.
RG Utrophin upregulation via microRNA repression as a therapy for DMD
$126,500.00 8/1/2013 7/31/2014 Year 3

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

James Shorter Ph.D.
RG Generating Therapeutic Protein Disaggregases for Amyotrophic Lateral Sclerosis
$100,000.00 8/1/2013 7/31/2014 Year 1
$100,000.00 8/1/2014 7/31/2015 Year 2
$100,000.00 8/1/2015 7/31/2016 Year 3

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

Hansell Stedman M.D.
RG Pattern Recognition Receptors in Muscular Dystrophy Pathogenesis and Therapy
$100,000.00 8/1/2013 7/31/2014 Year 1
$100,000.00 8/1/2014 7/31/2015 Year 2
$100,000.00 8/1/2015 7/31/2016 Year 3

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

Lee Sweeney Ph.D.
RG Modulation of calcium handling in mouse models of muscular dystrophy
$91,553.00 8/1/2013 7/31/2014 Year 1
$92,750.00 8/1/2014 7/31/2015 Year 2
$93,983.00 8/1/2015 7/31/2016 Year 3

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

Pittsburgh - University of Pittsburgh

Stephen Meriney Ph.D.
RG A novel calcium channel agonist as a treatment for LEMS
The proposed experiments will evaluate the effectiveness of a novel drug we have developed as a potential treatment for Lambert-Eaton myasthenic syndrome (LEMS). This compound is a potent calcium channel agonist, and we will characterize the effects of this novel drug on calcium channel gating in cell lines expressing the types of calcium channels present at motor nerve terminals, on transmitter release and muscle force generation at LEMS model mouse neuromuscular junctions, and on a battery of behavioral tests of muscle strength using LEMS model mice. This new drug could be used in isolation, but since the properties of this new drug are hypothesized to work synergistically with the most commonly used treatment approach for LEMS (3-4 diaminopyridine, DAP), we propose that our new drug may be best used in combination with DAP, both enhancing DAP effects and allowing DAP to be used at lower doses that greatly reduce potential side-effects.
from fat, protein, or carbohydrate may not be of equal value in preventing the cachexia, particularly if specific metabolic homeostatic pathways are perturbed in TDP-43 related disease, it is unclear from what source calories should be most encouraged. Knowledge from this project will be useful for guiding the best dietary strategies in disease. Clinical trials could readily be established to confirm the animal findings in human beings, allowing for the chance to readily improve patient outcomes.

**Peter Robin Hiesinger Ph.D.**

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

$100,000.00 8/1/2013 7/31/2014 Year 1

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

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

**Summary** Charcot-Marie-Tooth Disease type 2B (CMT 2B) is a sensory neuropathy caused by mutations in the gene rab7. This gene encodes a protein with a critical function in the degradation of intracellular debris in all cells. In patients, weakening and ‘dying back’ neuromuscular contacts in early adulthood lead to neuropathy symptoms, including severe sensory loss in limbs. Even though the gene is known, it is unclear how the mutations found in patients affect the gene’s function. The disease is dominant, i.e. one mutant copy and one normal version of the gene in patients are sufficient to cause the disease. It has therefore been proposed that the CMT 2B-causing mutations lead to increased function. We propose that this currently assumed reason for the dominance is incorrect. We have established the first animal model for CMT 2B using Drosophila as a model animal and primary rat neuronal culture for validation experiments. Our preliminary data show that dosage-dependent loss of rab7 gene function affects nerve cells before other cells in the body. Our findings explain the genetic dominance and reveal a particular sensitivity of nerve cells for a defect in debris removal. This discovery opens the door for an understanding and a potential therapy of CMT 2B based on the molecular manipulation of the underlying cause. Importantly, we suggest an increase of rab7 function as a therapeutic opportunity, in contrast to the currently suggested reduction of mutant gene function.

**Douglas Millay Ph.D.**

**Molecular control of mammalian myoblast fusion**

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

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

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

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

**Galveston - The University of Texas Medical Branch at Galveston**

**Premkumar Christadoss M.B.B.S**

**Complement siRNA gene therapy for myasthenia gravis**

$130,000.00 2/1/2013 1/31/2014 Year 2

$130,000.00 2/1/2014 1/31/2015 Year 3

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

**Houston - Baylor College of Medicine**

**Thomas A. Cooper M.D.**

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

Susan Hamilton Ph.D.

RG Central Core Disease and Mitochondrial Dysfunction

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

Summary Central Core Disease is a dominantly inherited myopathy characterized by metabolically inactive regions in the center of the muscle fibers. Mitochondria, the energy producers of the cell, are destroyed in the core regions. We will define the mechanisms of mitochondrial destruction in Central Core Disease and identify new therapeutic targets for CCD and other mitochondrial myopathies.

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

Muralidhar L. Hegde Ph.D.

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

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

Summary The genotoxicity of TDP-43, an hnRNP family protein primarily involved in RNA processing (but also binds to DNA) whose aggregation/toxicity have been etiologically implicated in Amyotrophic Lateral Sclerosis (ALS) is not investigated although the diseases associated with TDP-43 pathology accumulate significant genome damage. Our preliminary data demonstrate that: (1) TDP-43 is required for efficient DNA double strand break repair (DSBR) in neuronal cells; (2) TDP-43 is recruited to DSB sites to stably interact with DSBR proteins, in neuronal cells; (3) TDP-43 depletion markedly increases DSB accumulation and sensitized human cells to DSB-inducing agents; (4) Nuclear-specific depletion of TDP-43 in neuronal cells cause increased DSB damage. (5) A strong correlation between TDP-43’s nuclear clearance and the accumulation of DSBs in ALS-affected human postmortem spinal cord tissue. Based on these, we hypothesize that loss of nuclear TDP-43 in ALS, causes deficient repair of DNA strand breaks in neurons promoting cell death and thus deficient repair of DSBs is a key etiologic factor in ALS and other TDP-43 pathologies. These will be comprehensively tested in this project by pursuing three Specific Aims using state of the art biochemical, cell culture including primary neuronal culture) and mouse and human tissue. Achieving our goals will lead to a major paradigm shift in our understanding of ALS pathology will open up new avenues therapeutic interventions.

Houston - Methodist Neurological Institute

Stanley Appel MD

RG Immune Mechanisms in Amyotrophic Lateral Sclerosis.

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

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

Houston - The University of Texas Health Science Center at Houston

Radbob Darabi MD., Ph.D

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

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

Houston - The University of Texas Health Science Center at Houston

Mariah Rose Baker Ph.D.

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

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

Vasanthi Jayaraman PhD

**RG**
RNA Based Drugs for ALS

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

**UTAH**

**Salt Lake City - Sfida BioLogic, Inc.**

**John Paul Manfredi Ph.D.**

**RG** Evaluation in Mouse of Small Molecules to Treat Spinal Muscular Atrophy

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

**Salt Lake City - University of Utah**

**Nicholas Johnson M.D.**

**RG** A Longitudinal Study of Disease Progression in Congenital Myotonic Dystrophy

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

WASHINGTON

**Pullman - Washington State University**

**Buel D. Rodgers Ph.D.**

**RIG**  Washington Center for Muscle Biology, Exercise Physiology Phenotyping Core

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**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 Duchenne muscular dystrophy and many other myopathies. Thus, expanding resources for the WCMB, including those to assess exercise performance in mice, will help a large cadre of scientists with research and training interests in muscular dystrophy. Our long-term goal is to establish the WCMB as a premier muscle biology research unit in the country. The objective of this proposal is to establish an Exercise Physiology Phenotyping core with modular metabolic treadmills and digital running wheels. This equipment is particularly useful in assessing genetic models for muscular dystrophy and in evaluating gene and cell therapeutics in pre-clinical studies. Our justification is that this equipment will provide a necessary resource to the WCMB community and will further enhance muscular dystrophy research at several biomedical research institutions in the region. These efforts will not only assist center development, but will help foster intellectual exchange and technology transfer between academia and the state’s thriving biotech industry.

Seattle - Fred Hutchinson Cancer Research Center

**Zejing Wang M.D., Ph.D.**

**RG**  Gene therapy for treating cardiomyopathy in a dog model of DMD

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

Seattle - Seattle Institute for Biomedical and Clinical Research

**Brian Kraemer Ph.D.**

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

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

Seattle - University of Washington

Joseph A Beavo Ph.D.

**Proposal:** Mechanism of sildenafil action in muscular dystrophy

**Summary**

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

Gregory Block Ph.D.

**Mechanism of Wnt signaling in FSHD**

**Summary**

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

Jeffrey S Chamberlain Ph.D.

**AAV vectors for gene therapy of DMD**

**Summary**

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

**RG** RNA interference-based treatment of FSHD modeled in mice

$110,260.00 8/1/2013 7/31/2014 Year 2

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

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

Martin Childers PhDDO

**RG** Dystrophin-deficient cardiomyocytes for high-thruput drug screening

$160,000.00 8/1/2013 7/31/2014 Year 3

**Summary** Heart failure is a common and serious feature of Duchenne muscular dystrophy. The reason for this is because the heart muscle carries a genetic mutation that damages the normal operation of this all-important muscle. Our project will allow for the discovery of new drugs that might reverse, or prevent effects of the disease on the heart muscle in DMD patients. We will use two types of new technology to find potential new drugs. First, we will use a groundbreaking method called "cellular reprogramming". This method was first used to make stem cells out of skin cells from patients. In our project, we will first make stem cells from the skin cells of DMD patients, then we will use these stem cells to form beating heart cells. These newly "reprogrammed" heart cells will contain the same genetic mutation found in the patient’s own skin cells. Many thousands of reprogrammed cells can be generated to form identical heart cells, and these cells can be individually examined. This remarkable new technology will allow us to study how a genetic mutation affects the heart cells of a specific patient. The second method we will use is a drug discovery “platform” that can screen individual cells against thousands of drug compounds available for testing. By marrying these two incredible technologies, this project will allow for the first time, the ability to test new drugs directly on the heart cells from an individual patient without carrying any risk to the patient.

Stephen D. Hauschka Ph.D.

**RG** New Regulatory Cassettes for Treating Diseased Muscle Tissues

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

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

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

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

Deok-Ho Kim Ph.D.

**RG** Functional Restoration of Dystrophic Muscle using Bioengineered Cell Patches

$130,000.00 2/1/2013 1/31/2014 Year 1

$130,000.00 2/1/2014 1/31/2015 Year 2

$130,000.00 2/1/2015 1/31/2016 Year 3

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

**Morayma Reyes M.D., Ph.D.**

**RG**  Role of PDGF Receptor alpha signaling in DMD cardiac fibrosis

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