CALENDAR YEAR(s): 2016,2017,2018,2019

AUSTRALIA

Clayton - Monash University

Peter David Currie PhD

RG Using zebrafish congenital muscular dystrophy models to find novel therapies.

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Numerous studies have suggested that zebrafish genetic models of human diseases can recapitulate many aspects of the human pathology. This is particularly well documented for muscle wasting diseases where a number of genetic models of human muscular dystrophies have been identified by our laboratory. Specific to the aims of this project was the identification of a zebrafish mutation in the Laminin alpha 2 gene which is mutated in the most common form of congenital muscular dystrophy (CMD). We have used this zebrafish model to make observations on the mechanisms by why muscle cells die when they lack Laminin alpha2 protein. We now wish to understand this process better and will use the specific advantages of the zebrafish system to make observations that will lead to the identification of novel therapeutic approaches for the treatment of CMD. We have also developed methods to screen the zebrafish model of CMD to identify novel drug compounds and we will use these methods to find drugs that prevent the onset and progression of muscle waiting in this model. We hope these compounds will form the basis for the development of drugs to treat congenital muscular dystrophy.

Tamar Esther Sztal Ph.D

DG15 Evaluating therapies to improve muscle function in nemaline myopathy

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary Nemaline myopathies are congenital muscle diseases causing severe muscle weakness and low muscle tone. Typically patients exhibit skeletal muscle weakness and feeding difficulties in infancy, however, severe cases result in death at or before birth. The skeletal muscle weakness is non-progressive with survival to 30 years exceeding 80%. Therefore these patients suffer from muscle weakness and associated problems, such as respiratory insufficiency, throughout their life. There is no effective therapy for nemaline myopathy however many patients are self-administering supplements including tyrosine, following only anecdotal reports of their benefit. To screen for effective new therapies I have created zebrafish nemaline myopathy models caused by mutations in ACTA1 or NEB, accounting for 75% of severe cases. I have shown that these models accurately recreate the disease with fish containing nemaline bodies in their skeletal muscle and demonstrating decreased muscle function. Using the advantages of the zebrafish system for high-throughput chemical screening, I aim to identify effective novel therapies that are directly translatable to patients. I will evaluate existing compounds to determine their effect, if any, and to establish a baseline for new therapies to surpass. I will then test more than 1200 drugs to find the most effective at increasing muscle function and reducing disease severity.

Crawley - The University of Western Australia

Miranda Grounds Ph.D

RG16 Why does lipid accumulate in dysferlin-deficient muscles?

$95,370.00	8/1/2016	7/31/2017	Year 1

	$97,769.00	8/1/2017	7/31/2018	Year 2

Summary Dysferlinopathies are a form of muscular dystrophy that is caused by defects in the gene that makes a protein called dysferlin. Dysferlinopathies occur in humans, and in mice and zebrafish that are useful experimental models to study this disease. The reasons why progressive muscle weakness occurs in young adult humans are not clear and there is no effective treatment. We have shown that the dysferlin-deficient muscles contain many droplets of lipid (fat) within the muscle cells, and that fat cells (called adipocytes) replace the muscle cells over time. This will impair function and result in muscle wasting. This project investigates the molecular mechanisms for these striking lipid related changes. Aim 1 will describe how the key aspects of lipid metabolism are altered within dysferlin-deficient muscles. Aim 2 will examine dysferlin-deficient (and normal) muscle cells and adipocytes in tissue culture to test their lipogenic capacity, and cross-talk between combinations of these cells and their secreted products. Finally, in vivo studies in mice in Aim 3, will determine if the adverse clinical effects of glucocorticoids on dysferlinopathies is due to their capacity to enhance lipogenesis and adipogenesis. These combined studies will provide insight into the mechanisms that lead to the dystropathology in dysferlin-deficient muscles. This research aims to identify the best targets for therapy and accelerate future targeted drug therapy trials for dysferlinopathies.

Sydney - The University of Sydney

Joshua Burns Ph.D

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

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

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

Michael L.H. Huang Ph.D.

DG16 Targeting Mitochondrial Homeostasis in the Pathogenesis of Friedreich's Ataxia

$59,000.00	8/1/2016	7/31/2017	Year 1
$58,100.00	8/1/2017	7/31/2018	Year 2
$60,000.00	8/1/2018	7/31/2019	Year 3

Summary Friedreich's ataxia (FA) is a devastating neuro- and cardio-degenerative condition caused by a lack of a mitochondrial protein, frataxin. My interest in FA developed while being mentored by an established research program that has held continuous research funding from MDA USA since 2001. My research has resulted in 22 publications in the last 5 years, including 3 articles in PNAS (2 as 1st or equal-1st author). My studies identified mitochondrial defects in tissues lacking frataxin, leading to FA pathogenesis. As the heart and the nervous system rely heavily on mitochondria to fulfill their energy demands, they are most affected by its dysfunction. Thus, I will examine the extent that frataxin-deficiency disrupts mitochondrial homeostasis and the possibility of targeting this process as a therapy. Mitochondria constantly undergo fusion or division according to energy demands. As previous studies have shown proliferation of damaged mitochondria in frataxin-deficient cells, I will explore alterations in the ability for mitochondria to fuse or divide in our animal models of FA. Further, I will assess the pathological changes in the synthesis and maintenance of mitochondria in FA. Importantly, based on results in my first author article in the Am. J. Pathol. 2013;183:745-57, I will investigate the mechanism of how vitamin B3 can boost mitochondrial health to prevent the pathology in FA through its ability to affect mitochondrial dynamics, biogenesis and clearance.

BELGIUM

Gent - VIB vzw

Ludo Van Den Bosch Ph.D.

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

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

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

BRAZIL

São Paulo - Fundacao Faculdade de Medicina

Natassia Vieira Ph.D

DG Jagged1 as a genetic modifier of Dystrophin Deficiency

$60,000.00	8/1/2015	7/31/2016	Year 1
$60,000.00	8/1/2016	7/31/2017	Year 2
$60,000.00	8/1/2017	7/31/2018	Year 3

Summary Absence of functional dystrophin causes muscle degeneration in DMD, but additional factors involved in the pathogenesis remain poorly understood and represent an unexplored territory for therapy. Among the different animal models for DMD, the most similar to the human condition is the golden retriever muscular dystrophy (GRMD) dog. We identified milder affected GRMD dogs (here called escapers) clinically distinguishable from other affected dogs, despite the absence of muscle dystrophin, no utrophin upregulation and raised serum creatine kinase levels. With 3 independent approaches we found a new modifier gene, Jagged1, which can modulate the phenotype of these GRMD dogs. Jagged1 overexpression also rescues the dystrophic phenotype in the DMD zebrafish model. This candidate gene opens new possibilities for therapeutic approaches for DMD. Aiming to assess the therapeutic potential of Jagged1 we will a) determine the signaling pathways modulated by Jagged1 overexpression and b) evaluate the functional improvement in the mdx using AAV expression of Jagged1. We will establish mRNA expression profiles of normal and dystrophin-null muscle cells overexpressing Jagged1. This profile will give us new targets that can be used for therapeutic approaches. AAV represents a promising approach to control the expression of genetic modifiers aiming to improve function. The AAV delivery of Jagged1 will determine if transient overexpression improves disease pathology and muscle function.

CANADA

ONTARIO

Ottawa - Ottawa Hospital Research Institute

Rashmi Kothary Ph.D.

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

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

Summary A pathological hallmark of spinal muscular atrophy (SMA) is the loss of lower motor neurons in the spinal cord and corresponding muscular atrophy with subsequent paralysis and in most severe cases, death of young babies. Mutations in the survival motor neuron 1 (SMN1)

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

Michael A. Rudnicki PhD

RG15 Molecular regulation of satellite cell function

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Our overarching goal is to facilitate the development of Wnt7a as a protein biologic for the treatment of Duchenne Muscular Dystrophy (DMD). 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 stem cells. We have 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 stimulating the growth and function of these muscle stem cells resulting in the formation of increased numbers of myofibers. Here, we have found that dystrophin, the disease gene in DMD, is normally expressed in satellite cells, and its absence alters the function of satellite stem cells. 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 dystrophin-deficient satellite stem cell numbers to stimulate repair of skeletal muscle. Finally, we will assess the utility of Wnt7a as a drug for the treatment of DMD. This work will inform the development of Wnt7a as a therapeutic for the treatment of Duchenne Muscular Dystrophy.

Ottawa - University of Ottawa

Bernard Jasmin PhD

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

$84,600.00	5/1/2015	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.

Toronto - The Hospital for Sick Children

James Dowling M.D., Ph.D.

RG15 Drug Discovery for RYR1-related myopathies

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Myopathies caused by mutation(s) in the type 1 ryanodine receptor (RYR1), termed RYR1-related myopathies, are one of the most common muscle disease groups of childhood. In many cases, RYR1-related myopathies are associated with significant disabilities, including the need for a wheelchair for ambulation, severe spine curvature requiring surgery, and breathing difficulties necessitating the use of a ventilator. In addition, in some children, RYR1 mutations can result in premature death. Currently there are no therapies for these devastating myopathies. In this project, we will identify, develop, and validate new candidate therapeutics for RYR1-related myopathies. We will accomplish this by building on our novel drug development platform, which includes high throughput screening in C. elegans, rapid target validation in the zebrafish, and final testing in patient-derived myotubes. In a preliminary screen, we identified in C. elegans more than 100 possible candidate drugs. Using our proposed research protocol, we will validate and prioritize these novel drug targets using zebrafish and human cell models of the disease. We will also develop new worm and zebrafish models of the disease and screen them for new candidates. The end result will be the identification of drugs suitable for translation to patients, with the ultimate goal being the development of new therapies that will improve the quality and length of life for individuals with RYR1 myopathies.

QUÉBEC

Montreal - Jewish General Hospital/Lady Davis Institute for Medical Research

Colin Crist Ph.D.

RG ex vivo expansion of muscle stem cells with regenerative capacity

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Development of stem cell based therapies, either to modulate the behaviour of, or functionally replace, the pool of endogenous muscle stem cells participating in muscle regeneration, are potential therapeutic strategies for many disorders of skeletal muscle. Replacement of the endogenous pool of muscle stem cells is made difficult in part due to the limited number of donor muscle stem cells available, a problem that is exacerbated by the loss of stem cell behaviour and regenerative capacity when muscle stem cells are expanded ex vivo. Therefore, for stem cell based strategies to be realized as a therapeutic strategy for muscular dystrophies, a greater understanding of the molecular mechanisms governing the activity of muscle stem cells is needed. The long-term objectives of our research program are to further understand molecular mechanisms underlying muscle stem cell capacity to self-renew. We envision manipulating these mechanisms to accelerate stem cell based therapies for muscle disorders. In this proposal, we will enhance skeletal muscle stem cell regenerative capacity by pharmacological manipulation of a pathway regulating protein synthesis in the skeletal muscle stem cell.

Montreal - McGill University

Heather D. Durham Ph.D.

RG16 The role of nBAF chromatin remodeling complexes in ALS

$99,749.00	8/1/2016	7/31/2017	Year 1
$99,567.00	8/1/2017	7/31/2018	Year 2
$99,701.00	8/1/2018	7/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a complex disease with multiple causes, resulting in fatal loss of motor activity due to dysfunction and death of motor neurons. Multiple genetic mutations are linked to ALS, but the causes of most cases of disease that occur sporadically are unknown. ALS involves complex pathogenic cascades with widespread effects on cellular functions. The challenge is to identify and understand the key elements in those cascades to target for therapy. We have identified disruption of a protein complex that regulates expression of neuronal genes that 'make neurons neurons' and extend processes to connect to other neurons in the network controlling movement. These complexes are called nBAF chromatin remodeling complexes and key proteins of these complexes are lost in motor neurons in familial ALS caused by gene mutations and in sporadic ALS. Thus, we have identified a convergent mechanism that could be targeted to keep motor neurons connected in the network and functioning longer.

Montréal - Centre Hospitalier de l'Université de Montréal

Gary Armstrong Ph.D.

DG Synaptic mechanisms of neuronal dysfunction in genetic models of ALS

$59,890.00	8/1/2015	7/31/2016	Year 1
$59,890.00	8/1/2016	7/31/2017	Year 2
$57,890.00	8/1/2017	7/31/2018	Year 3

Summary Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder affecting motor and in some cases cognitive function. There is a single pharmacological treatment, Riluzole, which has limited therapeutic value. Part of the poor success of Riluzole, and many other clinical trials, can be attributed to our incomplete understanding of the synaptic abnormalities that arise following expression of causative mutations in genes such as superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TARDBP) and Fused in Sarcoma (FUS). In particular, we are lacking information about the earliest (‘pre-clinical’) events during cellular pathology, such as disruption of neuromuscular transmission, alterations in excitatory and inhibitory interneuron connectivity with motoneurons and changes in intrinsic excitability of motoneurons. We believe that many of these early pathological defects occur as a result of compensatory synaptic homeostatic plastic changes that are operating aberrantly. To investigate this concept I propose to use two model systems (zebrafish and mice). Addressing the questions proposed in brief below will help elucidate the molecular and pathological mechanisms of ALS pathology and complement our clinical trial of Pimozide.

Alex Parker Ph.D

RG15 TIR-1/Sarm1 mediated degeneration of motor neurons in ALS

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective death of motor neurons. Even though recent advances have discovered many of the genetic causes of ALS, it remains an incurable disease. To learn more about disease mechanisms and identify new therapeutic approaches we use the genetic system Caenorhabditis elegans, a nematode worm with powerful and rapid methodologies to model ALS. Using our C. elegans ALS models we discovered that the immune system is inappropriately activated via the protein TIR-1/Sarm1 and contributes to cell death. We propose that inactivating this TIR-1/Sarm1 signalling cascade, either genetically or with drugs will alleviate neuronal degeneration caused by mutant human proteins linked to ALS. We will investigate the TIR-1/Sarm1 pathway as a new therapeutic target for ALS in C. elegans and mouse models.

Christine Vande Velde Ph.D.

RG15 Misfolded SOD1 species and mitochondrial quality control in ALS

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis is a progressive and ultimately fatal neurodegenerative disease, characterized by the loss of specialized neurons that control voluntary muscle movement. The loss of these neurons, termed motor neurons, results in progressive weakening and ultimately paralysis of skeletal muscles. Affected individuals gradually lose their ability to move, speak, swallow and eventually breathe. The biological basis of specificity and the mechanism of how motor neurons are lost in ALS remains unknown. The second most common cause of familial inherited ALS and a portion of non-inherited sporadically occurring ALS cases are due to genetic mutations in superoxide dismutase 1. Mutant SOD1 protein adopts a non-normal/ misfolded structure, which leads it to associate with motor neuron mitochondria in an aberrant manner. Mitochondria are the power generators of the cells and neurons highly depend on them. The mechanism is unclear, but the association of non-normal SOD1 with these organelles causes damage. Mitochondria are equipped with a quality control system that normally safeguards against such insults. Thus, it is puzzling why damaged mitochondria increasingly accumulate and harm motor neurons in ALS.

Québec - CHU de Québec

Jack Puymirat M.D., Ph.D.

RG16 Elimination of toxic RNA in myotonic dystrophy brain

$93,215.00	8/1/2016	7/31/2017	Year 1
$96,319.00	8/1/2017	7/31/2018	Year 2
$89,003.00	8/1/2018	7/31/2019	Year 3

Summary Myotonic Dystrophy type 1 (DM1) is a multisystemic dominant disease caused by a CTG expansion in the DM1 gene. Pathogenic RNAs are retained in nuclear aggregates that sequester nuclear factors, ultimately leading to abnormalities of RNA maturation and clinical symptoms. There is increasing amount of evidence indicates that elimination of toxic RNA to inhibit its toxicity represents a valuable therapeutic strategy for DM1. Currently, there is no curative treatment available for this RNA-dominant disease. We will develop and evaluate antisense oligonucleotide technology including chemically modified antisense oligonucleotides and peptides oligonucleotides to abolish RNA toxicity by degrading CUGexp-RNA. Human DM1 neuronal cells derived from DM1 iPSC and animal models (DMSXL mice) are available. The incapacity of antisense oligonucleotides to effectively cross the blood-brain barrier indicate that other delivery methods will have to be considered for the treatment of DM1 CNS dysfunction and cognitive impairment, which have dramatic repercussion on the quality of life of patients. To this end, intraventricular injection in mice could resolve this issue and will be investigated. In human, intrathecal administration of ASO ISIS-SMNRx in patients with infantile spinal muscular atrophy cleared a phase III human study, showing the potential for this approach in DM1.

CHILE

Santiago - Institute of Biomedical Sciences, Faculty of medicine, University of Chile

Claudio A Hetz Ph.D

RG15 Targeting the ER stress sensor IRE1 to treat ALS

$98,000.00	2/1/2016	1/31/2017	Year 1
$98,000.00	2/1/2017	1/31/2018	Year 2
$98,000.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a progressive and deadly adult-onset motoneuron disease characterized by muscle weakness, atrophy, paralysis and premature death. The primary mechanism responsible for the progressive motoneuron loss in ALS remains unknown. Clues have been obtained from families with sporadic and familial ALS, which are accompanied by alterations in the folding of important proteins including SOD1, TDP43, FUS, among other factors. Perturbations of the protein folding functions performed at a subcellular organelle called the endoplasmic reticulum (ER) have been extensively suggested as a common factor driving motoneuron dysfunction in ALS. ER homeostasis alterations are one of the earliest defects observed in ALS models, which may drive initial disease stages that trigger loss of motor control and later death of motoneurons. We have obtained preliminary data supporting the involvement of a specific ER stress sensor in neuroprotection against experimental ALS. In this project we will develop a systematic approach and define for the first time the relative contribution of this main stress pathway in ALS models. We plan to use genetic and pharmacological approaches to target the ER stress factor in different mouse models of ALS and human ALS neurons, measuring the impact on disease progression, life span, and histopathological features. This work may lead to the design of novel therapeutic strategies to treat this fatal neuromuscular disease.

CYPRUS

Nicosia - CING - The Cyprus Institute of Neurology and Genetics

Kleopas A. Kleopa M.D.

RG Developing gene therapy for inherited neuropathy

$95,995.00	8/1/2015	1/30/2017	Year 3

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

FRANCE

Illkirch - CERBM GIE

Jocelyn Laporte Ph.D. Molecular Biology

RG Genetics and Physiopathology of Tubular Aggregate Myopathies

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

Summary Tubular aggregate myopathies (TAM) are characterized by progressive muscle weakness affecting the lower limbs and associated with muscle pain and cramps. On muscle biopsies, TAM show regular arrays of membrane tubules in muscle fibers. These aggregates can also be found as secondary features in various muscle disorders and accumulate in normal muscle with age. We recently identified a first gene implicated in primary TAM and encoding for a calcium sensor. Calcium triggers muscle contraction and is a key molecule for muscle

growth and differentiation. Consequently, the intracellular calcium flow has to be tightly regulated to ensure normal muscle function. We demonstrated that the identified mutations impact on the calcium level in muscle cells, but the exact disease mechanisms leading to muscle dysfunction and pain remain unknown. Therefore, we will analyze the nature, the origin and the impact of the tubular aggregates and the correlated calcium defects in cells and in an animal model. Moreover, we will test a potential therapeutic rescue using selected drugs acting on the calcium flow in both patient cells and animal model. As several patients of our TAM cohort do not harbor mutations in the previous gene, we will finally identify further TAM genes, potentially representing novel drug targets. This project will contribute to a better understanding of several myopathies and muscle aging.

ITALY

Novara - Department of Translational Medicine, University of Piemonte Orientale at Novara

Nicoletta Filigheddu Ph.D.

RG — Exploring the therapeutic potential of unacylated ghrelin for muscular dystrophy

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

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

Trieste - International Centre for Genetic Engineering and Biotechnology

Franco Pagani MD

RG15 — Exon Specific U1 snRNAs as a therapeutic approach for spinal muscular atrophy

$76,450.00	2/1/2016	1/31/2017	Year 1
$74,800.00	2/1/2017	1/31/2018	Year 2
$73,700.00	2/1/2018	1/31/2019	Year 3

Summary Spinal muscular atrophy (SMA) is a severe neuromuscular disease with no effective treatment. One of the most promising strategy is to act directly on the SMN2 gene and in particular on its "splicing" at the level of RNA, to force the gene to produce the missing protein. In this proposal, we intend to evaluate a novel therapeutic approach for SMN2 splicing correction based on small RNAs named "Exon Specific U1 snRNA" (ExSpeU1). In this project, we intend to evaluate the therapeutic and safety profile of ExSpeU1s in vivo in different mice SMA models. These studies will contribute to the development of an effective therapeutic approach for the treatment of SMA.

UNITED KINGDOM

Edinburgh - University of Edinburgh

Lyndsay Murray Ph.D

RG16 — Understanding and Exploiting the Therapeutic Time Window in Mouse Models of SMA

$94,268.00	8/1/2016	7/31/2017	Year 1
$98,611.00	8/1/2017	7/31/2018	Year 2
$99,295.00	8/1/2018	7/31/2019	Year 3

Summary SMA is a devastating motor neuron disease affecting primarily children, which is the result of degeneration of the cells known as motor neurons. This disease is caused by defects in a gene known as 'survival motor neuron 1', or SMN1. A number of therapeutic options have been proposed for SMA. Among the most promising of these, are strategies that are based on restoring the levels of SMN. Importantly, animal model trials revealed that while administration of the therapeutic can nearly rescue an individual from the disease when given before symptoms start, but the benefits are vastly reduced when therapies are given later, even at very early stages of the disease. As we will likely be treating patients after symptom onset, it is very important to understand why the benefits of therapeutics are so limited after symptoms have started, and find way in which to maximize the benefits during symptomatic stages of the disease. We aim to understand how long it takes for motor neurons to recover following restoration of SMN function and to understand how this process differs when the Smn gene is repaired at symptomatic stages of disease. We also plan to give other drugs, at the same time as repairing the SMN gene to see if they can add additional benefit when the gene is fixed at symptomatic stages of disease. This work will help us understand what limits the benefits of the therapeutics currently under development, and will investigate ways in which to make them work better.

Liverpool - University of Liverpool

Addolorata Pisconti PhD

RG Role of serine protease activity in the pathogenesis of muscular dystrophy

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Receiving a diagnosis of Duchenne muscular dystrophy (DMD) is devastating because there is currently no cure; moreover important aspects of what causes muscle loss in DMD remain to be understood. For example, it is not clear why the muscles of DMD children soon stop to regenerate themselves. We believe that this happens in part because cells that would normally regenerate the muscle when it is injured (called satellite cells) are exposed to a hostile environment generated by the continuous tissue damage caused by dystrophin loss. We have discovered that the levels of a family of proteins called serine protease inhibitors, which participate in the regulation of the response to tissue injury, are dramatically altered in the muscle of dystrophic mice. Furthermore, we found that addition of serine protease inhibitors to satellite cell cultures affects several satellite cell functions that are essential to ensure successful muscle regeneration. Thus, we propose to investigate whether the changes observed in mice also occur in children with DMD and to study the mechanisms through which serine protease activity regulates satellite cell regenerative capacity. This work is important because drugs targeting serine protease activity already exist and might be rapidly available in patients if proven to be useful in DMD models. Moreover, improving muscle regeneration will also improve the chance of success of gene therapies that are currently under development.

London - Institute of Neurology, University College London

Henry Houlden M.D., Ph.D., MRCP

RG15 Understanding early-onset neuropathies using genome and transcriptome sequencing

$98,357.00	2/1/2016	1/31/2017	Year 1
$99,212.00	2/1/2017	1/31/2018	Year 2
$90,582.00	2/1/2018	1/31/2019	Year 3

Summary Identifying the causative gene and understanding the mechanism is essential in developing effective disease therapies. Severe early-onset neuropathies are a diverse group where many patients remain undiagnosed and there are few treatments. We have worked extensively on a group of early-onset inherited neuropathies that range from severe early-onset pure neuropathy through to extreme phenotypes characterized by progressive bulbar palsy, respiratory problems and neuropathy that in the past were called Brown-Vialetto-Van Laere (BVVL) or Fazio-Londe syndrome after the describing physicians. In around 35% of BVVL and Fazio-Londe patients mutations in two riboflavin transporter genes have been identified where high-dose riboflavin supplements are an effective treatment, in some instances life-saving. We have built up a large series of genetically undefined inherited neuropathy patients. From this group we selected 30 genetically negative severe probands with additional DNA collected from parents and siblings as a trio/small family, with either white blood or skin cells, or muscle available for RNA/protein studies. In this proposal we plan to identify and characterize the disease genes in this group using whole genome sequencing to identify the disease associated variants, then use RNA sequencing to narrow down the mutation by identifying aberrant RNA splicing or reduced RNA expression (lost function) and subsequently characterize the disease genes in mammalian and patient cells

London - University College London

Federica Montanaro Ph.D.

RG Defining the role of impaired Hedgehog signaling in DMD

$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.

Oswestry - Robert Jones & Agnes Hunt Hospital

Glenn Eric Morris D. Phil.

RIG The MDA Monoclonal Antibody Resource for Neuromuscular Disease

$51,424.00	2/1/2016	1/31/2017	Year 1
$46,546.00	2/1/2017	1/31/2018	Year 2

Summary The MDA Monoclonal Antibody Resource was funded by MDA until Oct 31st, 2013 and by that date nearly all of the most popular hybridomas had been transferred to the Iowa Hybridoma Bank (DSHB) and are also available from that source. We have continued to respond to antibody requests and to maintain the website for nearly two years since MDA funding ceased. We are now applying for further funding for 2-years (part-time) in order to achieve additional specific aims, including making available new antibodies developed recently. The antibodies (over 300) are widely used in clinical trials of Duchenne MD therapy, in therapy trials on animals and in basic research to find novel therapies.

Portsmouth - University of Portsmouth Higher Education Corporation

Darek Gorecki Ph.D.

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

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

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

UNITED STATES

ALABAMA

Birmingham - Southern Research Institute

Mark Suto Ph.D.

RG Development of small molecules active at disease onset in ALS

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

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

Birmingham - The University of Alabama at Birmingham

Michael Miller Ph.D.

RG15 Postnatal Origin of Amyotrophic Lateral Sclerosis

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2

$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron degeneration. The average age of symptoms is in the mid-fifty's. Despite great efforts, nearly all tested ALS therapies have failed to provide benefits in clinical trials. The major limitation in developing effective therapeutics for ALS is inadequate understanding of the causal mechanisms. We have been studying the Vapb/ALS8 protein because Vapb loss causes ALS. Investigating the "normal" Vapb function is providing valuable clues to ALS pathogenesis. Our studies in worms, flies, mice, and humans support the model that Vapb is a circulating factor important for skeletal muscle energy metabolism. Preliminary data in this proposal suggest that Vapb is critical during postnatal reproductive development (i.e. circa puberty) and early adulthood. These data have important implications for ALS therapies, which could fail because disease onset occurs much earlier than clinical detection. Indeed, we have identified compensatory mechanisms that protect against muscle energy deprivation during aging. Here we will use worms and mice to further investigate Vapb and identify early biomarkers. Early detection is the key to eliminating disease. As Vapb is a circulating hormone like insulin, Vapb injections could be therapeutic.

Marek Napierala Ph.D.

RG16 Oligonucleotide-mediated therapy of Friedreich's ataxia

$92,907.09	8/1/2016	7/31/2017	Year 1
$94,107.09	8/1/2017	7/31/2018	Year 2
$92,504.03	8/1/2018	7/31/2019	Year 3

Summary Friedreich's ataxia (FRDA), a severe progressive neurodegenerative disorder, is caused by an increasing number of specific DNA sequences, termed GAA repeats, that are present in the Friedreich's ataxia gene (FXN). This error in DNA causes a block in the flow of the information from DNA to RNA, and ultimately leads to a deficiency of the final FXN product, a protein called frataxin. All FRDA patients produce a small amount of frataxin that functions, yet this amount is insufficient to maintain healthy cells. In the proposed project we will take a novel approach aimed to increase the amount of frataxin in patient cells. We will use molecules called oligonucleotides, small specific DNA fragments that can spontaneously enter diseased cells, locate frataxin RNA, and stabilize this important intermediate to increase its "molecular lifespan" in patient cells. This strategy does not increase production of the frataxin RNA, which has proven to be difficult, but instead allows existing frataxin RNA to be available longer for the process of frataxin protein production. We predict that the result of oligonucleotide treatment will be an increased amount of frataxin protein in FRDA patient cells. In summary, this work is contributing to the development of novel strategy to treat frataxin deficiency in Friedreich's ataxia.

ARIZONA

Phoenix - Dignity Health dba St. Joseph's Hospital & Medical Center

Rita Sattler

RG Role of synaptic dysfunction in C9orf72-mediated pathogenesis

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary The goal of this proposal is to study cellular and molecular mechanisms of disease pathogenesis induced by the novel C9orf72 mutation found to be highly prevalent in ALS patients. In specific, we will test the hypothesis that mutant C9orf72 leads to significant changes in the cellular structure of fine projections of neurons, so called axons and dendrites, which are important for the transmission of information from one cell to another. Preliminary data suggest that there is a dysfunction of the dendritic synapse, a specialized structure along those neuronal processes where signal transmission occurs, but also where

memories are formed and lost, as is the case during cognitive impairment, as frequently observed in C9orf72 ALS patients. Using C9orf72 ALS patient-derived adult induced pluripotent stem (iPS) cells we will study the mechanisms that alter synaptic proteins in regards to expression, localization and subsequently synaptic function. All human iPS cell culture in vitro studies will be followed up and validated with in vivo analyses of newly developed C9orf72 mouse models. The identification of these novel disease pathways is crucial for understanding C9orf72 disease pathogenesis and for the development of future therapeutics for disorders characterized by the C9orf72 mutation.

Tucson - Arizona Board of Regents, University of Arizona

Henk Granzier PhD

RG Improving Muscle Function in Nebulin-based Nemaline Myopathy

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

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

Daniela Zarnescu Ph.D.

RG16 Metabolic dysregulation in ALS

$99,951.00	8/1/2016	8/1/2017	Year 1
$99,881.00	8/2/2017	8/1/2018	Year 2
$99,986.00	8/2/2018	8/1/2019	Year 3

Summary Amyotrophic Lateral Sclerosis (ALS) is a fatal neurological disorder characterized by motor neuron loss and muscle atrophy. With the recent identification of cellular aggregates containing TDP-43 and the discovery of TDP-43 mutations in patients, this protein has emerged as a common denominator for the majority of ALS cases. We have developed a fruit fly model of ALS based on TDP-43, which exhibits alterations in locomotor function and lifespan that are remarkably similar to the human disease. Using this model we have identified specific alterations in the cellular metabolic pathways that govern energy production in motor neurons affected by ALS. These findings suggest increased glucose utilization and defects in the way mitochondria, the cell's power plants are utilizing fatty acids for energy production. Preliminary studies in the fly model show that locomotor defects are rescued by improving glucose or lipid metabolism in motor neurons via genetic manipulation or specific dietary changes. Given the presence of comparable alterations between fly models and ALS patients we propose to use molecular and genetic tools together with dietary intervention to restore cellular energetics. Our studies in the fly will be validated in patient derived motor neurons, which will help establish the feasibility of developing therapeutic strategies aimed at restoring defects in energy production in affected motor neurons and glia.

CALIFORNIA

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

Ricardo Anibal Maselli M.D.

RG Replacement Therapy for Congenital Deficiency of Endplate Acetylcholinesterase

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Congenital endplate acetylcholinesterase (AChE) deficiency is a human disease characterized by fatigable muscle weakness that results from an abnormal transmission of electrical signals through the neuromuscular junction (NMJ). Congenital endplate AChE deficiency is caused by a genetic deficiency of the collagen Q (ColQ), which is the protein that anchors AChE to the NMJ. There is currently no effective treatment for human deficiency of endplate AChE. The main goal of this project is to test the therapeutic effect of introducing genetically modified masenchymal stem cells (MSCs) expressing high levels of ColQ into engineered mice that lack ColQ and have severe muscle weakness. The ultimate goal of the project is to implement therapies for human deficiency of endplate AChE based on the replacement of ColQ at the NMJ.

La Jolla - Ludwig Institute for Cancer Research Ltd

Don Cleveland Ph.D.

RG Mechanisms underlying neurotoxicity caused by ALS-linked mutations in FUS/TLS

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative disorder, characterized by the selective loss of motor neurons. Recent breakthrough discoveries of mutations in the transactive response DNA-binding protein (TDP-43) and fused in sarcoma/translocated in liposarcoma (FUS/TLS) as causative of ALS and Frontotemporal dementia (FTD), combined with the abnormal aggregation of these RNA binding proteins, suggest that perturbations in both RNA and protein homeostasis may contribute to neurodegeneration. Unresolved is whether pathogenesis in TDP-43- or FUS/TLS-mediated disease results from a gain of toxic property(ies) associated (or not) with their cytoplasmic inclusions and/or a loss of nuclear function of either protein. To evaluate the contribution of gain of toxic property(ies) of FUS/TLS and the role of its aggregation in ALS/FTD, disease-specific changes in FUS/TLS protein interaction will be identified using new FUS/TLS-mediated ALS mouse models which develop adult-onset progressive neurodegeneration. The toxic potency of the aggregation-prone capacity of FUS/TLS will be tested using synthetic FUS/TLS fibrils in both cellular and mouse models. This multi-pronged approach is designed to resolve the nature of FUS/TLS interacting partners that are altered in disease as well as the contribution of FUS/TLS aggregation to pathogenesis, thus providing potential directions for therapies.

La Jolla - Sanford Burnham Prebys Medical Discovery Institute

Pier Lorenzo Puri M.D.

RG16 Role of fibroadipogenic progenitor subpopulations in Duchenne Muscular Dystrophy

$99,655.00	8/1/2016	7/31/2017	Year 1
$99,655.00	8/1/2017	7/31/2018	Year 2
$99,655.00	8/1/2018	7/31/2019	Year 3

Summary Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy, for which there is no available cure. Pharmacological control of disease progression is a suitable strategy to slow down the functional decline of dystrophic muscles. Targeting key pathogenic events of disease progression, such as transition from compensatory regeneration to fibrotic and fatty infiltration, is currently the focus of therapeutic interventions. This proposal will investigate the functional interplay between key cellular determinants (including muscle stem cells and component of their "niche") of skeletal muscle ability to regenerate upon acute injury or undergo fibrosis and fat deposition during chronic diseases, such as DMD. We have established a technological platform and experimental setting that allow to identify and functionally characterize discrete subpopulations of fibro-adipogenic progenitors (FAPs) in mouse models of acute regeneration, DMD progression and macrophage depletion, and will exploit HDAC inhibitors (a treatment currently in clinical trial with DMD boys) to determine the impact of pharmacological interventions on the relative amounts and biological activity of FAP subpopulations.

Alessandra Sacco Ph.D.

RG15 Role of STAT3 signaling in Duchenne Muscular Dystrophy

$99,279.00	2/1/2016	1/31/2017	Year 1
$99,279.00	2/1/2017	1/31/2018	Year 2
$99,279.00	2/1/2018	1/31/2019	Year 3

Summary In Duchenne muscular dystrophy (DMD) the diseased microenvironment plays a deleterious role on muscle stem cell (MuSC) function, thus impairing tissue repair. The goal of these studies is to develop pharmacological approaches to target these negative interactions. We hypothesize that by modulating the sensitivity of MuSC to the diseased microenvironment, we can promote their effective expansion in dystrophic muscle, thus restoring their ability to repair the damaged tissue and delaying disease progression. This proposal builds on our recent proof of principle study showing that transient inhibition of STAT3 signaling promoted MuSC expansion and improved tissue repair in dystrophic mice. In an effort to move this study forward, we will test STAT3 inhibitors (STAT3i) that have already been extensively characterized in preclinical studies, thus accelerating translation to the clinic. We will test the hypothesis that the STAT3 pathway is a major mediator of the deleterious effects of the dystrophic microenvironment on MuSC function. We will assess the effects of STAT3i on these cell-cell interactions and disease progression. For these studies we will utilize the mdx/mTR mouse, a model of DMD we recently developed that closely recapitulates the human DMD disease. These studies would improve our understanding of the progressive decline in tissue maintenance in DMD and develop strategies to ameliorate the disease phenotype that would find applications for regenerative medicine.

La Jolla - The Salk Institute for Biological Studies

Pradeep Reddy Dubbaka Venu Ph.D.

DG15 Preventing transmission of mitochondrial myopathies through heteroplasmic shift

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary Mitochondrial myopathies are a group of devastating diseases caused by mutations in mitochondrial DNA (mtDNA). Currently genetic counseling and preimplantation genetic diagnosis (PGD) are the best options to prevent the transmission of mitochondrial diseases. In this project we aim to prevent the transmission of mitochondrial myopathies by the selective elimination of mutated mtDNA present in the oocytes. The technique is based on the introduction of nucleases (molecular scissors) into oocytes that enter into mitochondria and specifically identify and eliminate the mutated mtDNA. The feasibility of this approach was recently demonstrated by using nucleases in the mouse embryos where the

transmission of targeted mtDNA to next generation was successfully prevented. A similar strategy will be undertaken in human oocytes from mitochondrial disease patients to selectively target and eliminate the mutated mtDNA. The assessment of safety and efficacy of this approach will be beneficial in moving this technology to the clinic in the near future. The results arising from this proposal may potentially lead to the eradication of mitochondrial myopathies.

La Jolla - The Scripps Research Institute

Matthew Disney Ph.D.

RG15 Designer Small Molecules to Manipulate Disease-Causing RNA Repeats

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Myotonic dystrophy type 1 (DM1) is a genetic disease characterized by multisystemic wasting of muscle function, including organ wasting that leads to cardiac disease, respiratory impairment, cataracts, and a host of other significant problems. Likewise, amyotrophic lateral sclerosis (ALS) is a progressive, degenerative disorder of motor neurons, resulting in muscle atrophy and paralysis. Up to 50% of ALS patients also develop abnormalities in behavior, language and personality. Both diseases are caused by a toxic biomolecule. No effective treatment is available for ALS or DM1. Our proposed work focuses on the optimization of two drug-like compounds, one that ameliorates DM1-associated defects in patient-derived cell lines and a mouse model and another that improves ALS-associated defects in patient-derived iNeurons and is blood-brain barrier penetrant. We will engender our compounds with the ability to remove the toxic biomolecule from disease-affected cells. We will also study the selectivity of our compounds in patient-derived cells and mouse models using novel and innovative methods developed by our laboratory. These studies can identify potentially silent off-targets, which could cause side effects. Since any therapeutic for DM1 or ALS would, in principle, be given to a patient for the course of their life, these studies and tools to investigate off-targets are critically important. Collectively, our studies will accelerate treatments for DM1 and ALS patients.

Los Angeles - Cedars-Sinai Medical Center

Robert Baloh M.D., Ph.D.

RG MFN1 augmentation to suppress toxicity in a novel mouse model of CMT2A

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Charcot-Marie-Tooth (CMT) disease is split into two forms, demyelinating (CMT type 1) and axonal (CMT type 2). Mutations in MFN2 are the most commonly identified cause of CMT type 2, but the mechanism of how altered function of this mitochondrial protein causes nerve damage remains unknown, and no effective animal models exist for the disease. Our lab found that in an in vitro model of the disease, overexpression of MFN1 was able to correct the mitochondrial defects and suppress axon degeneration. We developed a new transgenic mouse model of CMT2A, as well as a new transgenic mouse which overexpresses MFN1. Our goals in this proposal are to (i) characterize the new MFN2 mutant model, and (ii) determine whether increasing MFN1 levels in an animal model can suppress behavioral and pathologic features of the disease. This will provide proof of concept that increasing MFN1 levels is a viable therapeutic strategy in CMT2A.

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

Steve Cannon MD, PhD

RG15 A mouse model for exercise-induced weakness in hypokalemic periodic paralysis

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Hypokalemic periodic paralysis (HypoKPP) is a rare inherited disorder of skeletal muscle in which affected individuals have transient episodes of weakness, lasting hours to days. The attacks of weakness are usually provoked by stress, carbohydrate-rich meals, shifts in blood potassium levels, or follow a period of strenuous physical exertion. The cause for exercise-induced attacks is unknown and therefore therapeutic options are severely limited. Several gene defects have been identified in periodic paralysis, and with prior support from the MDA, we have created genetically modified mice with point mutations in sodium or calcium channel genes and that recapitulate the susceptibility to weakness when challenged with carbohydrate or shifts in potassium. New preliminary studies have revealed a profound loss of force within minutes of recovery from exposure to high carbon dioxide levels. We propose this maneuver is a surrogate for the exercise-induced attacks of weakness that occurs in patients. The discovery of this robust assay in the mouse model provides a unique opportunity in which we will investigate the mechanistic basis for exercise-induced weakness in periodic paralysis, will strategically select drugs that may block this process, and will test the potential of these drugs to foreshorten or prevent attacks of periodic paralysis.

Rachelle H. Crosbie-Watson Ph.D.

RG16 Improving cell adhesion to protect the dystrophic cardiac sarcolemma

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Our proposal will determine whether application of sarcospan treatment for Duchenne Muscular Dystrophies will affect cardiomyopathy disease progression in relevant murine models. We will investigate molecular mechanisms of cardiomyopathy in regulating adhesion and determine whether sarcospan is cardioprotective.

Melissa Spencer Ph.D.

RG15 Therapeutic Development of Osteopontin Inhibitors as Anti-Fibrotics for Duchenne

$173,663.00	2/1/2016	1/31/2017	Year 1
$143,663.00	2/1/2017	1/31/2018	Year 2
$133,663.00	2/1/2018	1/31/2019	Year 3

Summary The studies are designed to support the development of osteopontin inhibitors to treat Duchenne muscular dystrophy.

Los Angeles - USC/University of Southern California

William King Engel

RRG IBM Restricted Research Funding

$35,000.00	1/1/2016	12/31/2016	Year 2

Summary

Justin Ichida Ph.D.

RG The Role of C9ORF72 Protein Function in Amyotrophic Lateral Sclerosis

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary A massive expansion of a repetitive DNA sequence in a gene called C9ORF72 is the most common cause of ALS. Determining the mechanism of C9ORF72 pathogenesis is crucial for developing effective therapeutics. Recent studies have proposed different mechanisms to explain the motor neuron degeneration resulting from the C9ORF72 repeat expansion, mostly focusing on a gain of toxicity from the expanded C9ORF72 RNA itself or repetitive proteins generated from it. However, the mechanism of neurodegeneration remains unclear. To investigate the role of C9ORF72 protein in ALS pathogenesis, we used "cellular reprogramming" to generate motor nerve cells in the petri dish from the blood of C9ORF72 ALS patients. Using this approach, we have determined that an understudied mechanism, the loss of C9ORF72 protein function, plays a major role in inducing C9ORF72 ALS motor neuron degeneration. We find C9ORF72 ALS motor nerve cells express reduced levels of C9ORF72 protein and that restoring normal expression rescues their survival. In this project, we will use our human motor neuron model and biochemical and genetic methods to 1) Definitively show that the loss of C9ORF72 protein function plays a key role in C9ORF72 ALS, 2) Identify the properties of C9ORF72 that are critical to its protective function in ALS motor neurons and 3) Determine if C9ORF72 is a Rab guanine exchange factor. This work will establish C9ORF72 protein as a key therapeutic target.

Kim Staats Ph.D.

DG15 The Role of SEC14L5 in ALS

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary ALS is a relentlessly fatal disease driven by motor neuron cell death. Understanding the effect of mutations in ALS is necessary for development of therapeutic strategies. The Ichida lab has developed a method to systematically identify new mutations that cause sporadic ALS (PhenoSeq). Detected mutations are studied in-depth in induced motor neurons (iMNs), specifically derived from the patient. We propose the investigation in iMNs of an already identified mutation, which function is closely related to that of the largest genetic contributor of ALS, and its role in ALS. This mutation is in the gene SEC14L5 and has not yet been linked to ALS. Interestingly, besides the detection of a mutation in this gene by the PhenoSeq pipeline, the function of this gene is closely associated to the newly discovered function of the largest genetic contributor to ALS, C9ORF72, also by the host lab. This research will provide a unique analysis of the identified mutation in SEC14L5 and its role in human motor neuron death in ALS, which will increase the understanding of ALS disease mechanisms and facilitate the development of novel therapeutic strategies.

Palo Alto - Palo Alto Veterans Institute for Research

Antoine de Morrée Ph.D.

DG Towards FSHD Therapeutics: Understanding Polyadenylation Site Choice

$60,000.00	8/1/2015	7/31/2016	Year 1
$60,000.00	8/1/2016	7/31/2017	Year 2
$60,000.00	8/1/2017	7/31/2018	Year 3

Summary FSHD (Facioscapulohumeral Muscular Dystrophy) is a muscle disease for which no treatment exists. The disease is caused when muscle cells inadvertently make a toxic protein, called Dux4. There are several steps in the production process of this protein, and in theory at each step there is a possibility to intervene and block production. Such an intervention would stop the muscle from making the toxic protein and gradually allow for detoxification. An essential step towards producing the protein is making stable mRNA. Each mRNA molecule functions as a recipe for making a particular protein. Healthy individuals do not make the Dux4 protein, because they do not make stable mRNA for it. It turns out that FSHD patients have a mutation that allows cells to stabilize those mRNA molecules that are needed to make Dux4 protein. They do so using a process called polyadenylation. Therefore, any intervention that would prevent stabilization of these mRNA molecules would

be a potential treatment for FSHD. The goals of the experiments described in this proposal are to understand how cells regulate the polyadenylation of mRNA molecules, and to develop an intervention strategy that would prevent stabilization of only those mRNA molecules needed to make Dux4. We will directly test whether blocking this process leads to a reduction of Dux4. These studies have the potential to lead directly to new treatments that will reduce the toxicity in the muscles of patients with FSHD.

Thomas Rando M.D., Ph.D.

RG Non-invasive imaging of disease progression and treatment response in mdx mice

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Therapeutics for muscular dystrophies remain largely symptomatic. The use of cell or gene therapy holds the promise of a cure, but there remain many hurdles to translate advances in the laboratory to clinical trials. The main areas of focus of the studies of this proposal are the development of mouse models, so-called "reporter mice", that will be of greatest value for testing cell and gene therapy in terms of altering the progression of the disease. In the initial studies, we will use one of these reporter mice to test for the efficacy of viral gene therapy as means to halt the progression of the disease in the mouse model of Duchenne muscular dystrophy. In a second set of experiments, we will test for the effectiveness of a different reporter mouse strain to provide complementary information about disease progression. Together, the reporter mouse strains will be of tremendous value to the scientific community for testing all kinds of experimental therapeutic approaches for the treatment of muscular dystrophies.

Palo Alto - Stanford University

Michele Calos Ph.D.

RG16 DNA-mediated gene therapy for limb girdle muscular dystrophy

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Muscular dystrophies are caused by mutations in muscle genes. The most direct way to correct muscular dystrophy is to use gene therapy to supply a correct copy of the mutated gene to the affected muscle cells. While viruses are being developed for use in gene therapy, they have many limitations. A simpler alternative that has been shown to be effective in muscle is delivery of “naked” DNA through the bloodstream in such a way that it can enter muscle cells and become permanently incorporated in them. This approach may be especially effective in the limb girdle muscular dystrophies, where a more limited number of muscles need to be treated, and the heart and diaphragm are usually not involved. We will develop an effective DNA-mediated gene therapy method in mouse models of limb girdle muscular dystrophy 2B and 2D, which are deficient in dysferlin and alpha-sarcoglycan proteins, respectively. We possess the necessary mouse models, plasmids, antibodies, and assays and have already verified that gene delivery to limb muscles is effective and genomic integration is beneficial. We will demonstrate improvement in locomotor activity and muscle histology as a result of delivery of the therapeutic genes. This delivery method has been shown to be effective in large animals, so a rapid pathway to the clinic is available following these proof-of-principle studies in disease model mice.

Aaron Gitler Ph.D.

RG Defining a novel role of profilin 1 in ALS pathogenesis

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

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

Pasadena - California Institute of Technology

David Chan MD/PhD

RG	Mitochondrial dynamics as a protective factor in mitochondrial myopathies			
	$84,600.00	5/1/2015	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 Francisco - The Regents of the University of California, San Francisco (Contracts & Grants)

Douglas B Gould Ph.D.

RG	Genetic and Pharmacologic Manipulation of COL4A1: Potential Relevance to MDC1A			
	$84,600.00	5/1/2015	4/30/2016	Year 1
	$84,600.00	5/1/2016	4/30/2017	Year 2
	$84,600.00	5/1/2017	4/30/2018	Year 3

Summary We have recently discovered that patients and mice with mutations in the collagen type IV alpha 1 gene (COL4A1) have highly variable neuromuscular disease. Because COL4A1 is a major component of basement membranes we hypothesize that myopathy caused by COL4A1 mutations may be mechanistically similar to myopathy caused by mutations in laminin alpha 2 (LAMA2). LAMA2 is another important component of muscular basement membranes and mutations in LAMA2 cause MDC1A. In this project we will compare the pathology caused by COL4A1 and LAMA2 mutations. Irrespective of the specific outcome, we will seek to identify genetic loci and cellular pathways that modify Col4a1–induced

disease and will test the efficacy of bioactive small molecules as potential therapeutic drugs to ameliorate myopathy.

Daniel Kopinke Ph.D.

DG Ciliary Regulation of Muscle Regeneration

$50,760.00	5/1/2015	4/30/2016	Year 1
$50,760.00	5/1/2016	4/30/2017	Year 2
$50,760.00	5/1/2017	4/30/2018	Year 3

Summary Skeletal muscle has a robust ability to heal after wounds. Muscle repair depends on two distinct stem cells found within the muscle, the satellite cells, which give rise to new myofibers, and the fibro/adipogenic progenitors (FAPs), which coordinate satellite cell behavior. In investigating how FAPs help muscles repair injuries, I discovered that FAPs are the only cells in the muscle that possess primary cilia. Primary cilia are structurally similar to the cilia that propel paramecia through water, but do not move. Instead primary cilia act much like antennas to transmit signals from other cells. The ability of muscle to recover from wounds is compromised with old age and in certain chronic diseases, such as Duchenne muscular dystrophy (DMD). In these conditions, stem cells fail to restore muscle function after injury and the muscle is replaced with fibroblasts and fat. I found that interfering with FAP cilia in mice inhibits the replacement of muscle with fat. This project builds off of these findings to elucidate how cilia control FAP function during muscle injury repair, what signals these cilia sense, and whether I can use drugs to manipulate FAP ciliary signaling to prevent fibrosis and fatty infiltration. This work will illuminate how cilia control stem cell behavior and how ciliary signaling controls FAP function in muscle regeneration. I will use this understanding to assess whether modulating ciliary signaling in FAPs may provide a novel therapy for DMD.

COLORADO

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

Bradley Olwin Ph.D.

RG15 Enhancing Regeneration to Improve Dystrophic Muscle

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Skeletal muscle function progressively declines in the majority of muscular dystrophies accompanied a reduction and eventually a failure to regenerate. Exhaustion of muscle stem cell replicative capacity is widely assumed to be responsible for the decline in muscle regeneration despite the lack of data supporting this hypothesis. A cell surface protein that functions as an adhesion receptor (Syndecan-3) appears required to maintain muscle stem cells in their niche or normal home sandwiched between the muscle fiber and connective tissue. When the gene for Syndecan-3 is deleted in the mouse, muscle stem cells leave their niche but are found in the muscle interstitium, where the cells proliferate as myoblasts or muscle progenitor cells. We bred mice lacking the Syndecan-3 gene to mice lacking dystrophin and found that muscle function in resultant mice was dramatically improved, with inflammation and fibrosis reduced and voluntary exercise similar to normal, wild type mice. We believe that the interstitial myoblasts repair dystrophic muscle efficiently ameliorating the dystrophic phenotype and that the muscle stem cells are impaired but not intrinsically defective. We propose to test if inhibition of Syndecan-3 will improve dystrophy other muscular dystrophies and begin to develop therapies to inhibit Syndecan-3.

Fort Collins - Colorado State University

Steven Matthew Markus Ph.D.

RG15 THE MOLECULAR PATHOLOGY OF MOTOR NEURON DISEASES

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Intracellular transport is a fundamental and critical process whereby various cargoes are delivered to appropriate sites where they are needed to promote cell growth, maintenance, and division. Defects in this process compromise the survival and maintenance of several cell types, most notably motor neurons that communicate information from the spinal cord to muscles throughout the body, some of which can be as long as one meter. A critical component of the transport machinery is a family of motor proteins that carry cargoes along long polarized filaments called microtubules. One of these motors, dynein, is entirely responsible for the transport of cellular cargo such as organelles and proteins toward the cell center. Mutations in genes that encode dynein components are linked to several types of motor neuron diseases, including spinal muscular atrophy and amyotrophic lateral sclerosis. The underlying defects that give rise to these diseases are not known. This presents a significant barrier to developing effective and targeted therapeutics. The goal of our research is to apply a set of rapid, economical, and rigorous experimental strategies to dissect the molecular basis for dynein dysfunction in various types of motor neuron diseases. In addition to providing valuable insights into the pathology of these diseases, our research will lay the necessary foundation to identify effective, targeted therapies that have the potential to alleviate symptoms in affected patients.

DISTRICT OF COLUMBIA

Washington - Children's Research Institute (CNMC)

Yetrib Hathout Ph.D.

RG Development and validation of pharmacodynamic biomarkers for Duchenne.

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Currently there is no effective treatment for Duchenne muscular dystrophy (DMD) except use of corticosteroids that delays muscle inflammation for couple years but cannot cure the diseases and often results in adverse side effects. Recent and promising therapeutic strategies such as those aiming to restore the missing dystrophin protein and anti-inflammatory drugs with little to no side effects are being developed. However approval by a regulatory agency to test these drugs in DMD patients has been delayed due to the lack of tools to monitor drug efficacy. The only monitoring tool available today is the 6 min walk test, how far a patient can walk in 6 min. Unfortunately this test has proven to be challenging to preform by young children and also cannot be used for children who are about to lose or have lost ambulation. In this research project we propose to develop a panel of biomarkers detectable in blood circulation that are associated with DMD disease progression and, more importantly, can indicate if a drug is doing what it supposed to do or failed. We have already identified several biomarkers for DMD and we will test if these biomarkers will respond to new generation drugs for DMD.

Terence Anthony Partridge Ph.D.

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

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

Summary For Duchenne muscular dystrophy (DMD), exon-skipping is one of the most promising approaches. It works by excluding the mutated part of the gene from the messenger RNA copy (the blueprint) of the gene that is translated within the muscle fiber into the dystrophin protein. This is done by giving an antisense oligonucleotide, short length of a DNA-like structure, that masks the sites that are normally used to include this part of the

gene. Removal of this damaged region leads, in most cases, to the production of a slightly smaller dystrophin protein that retains part of the function of normal dystrophin, which should transform the clinical picture from the severe pathology associated with DMD to a milder disease that resembles Becker muscular dystrophy. The main problem with the present antisense reagents is that they do not readily enter all muscles , least of all the heart muscle. We propose to test a new chemical form that appears to enter heart and skeletal muscle. We will test this on a dystrophic mouse that has a mutation in the region of the dystrophin gene where many human DMD mutations occurs. This will allow us to test a larger variety of antisense agents than is possible with the dystrophic mouse we have used up to now.

Washington - The George Washington University

Linda L Kusner Ph.D

RG16 INHIBITORS OF APOPTOSIS IN MYASTHENIA GRAVIS.

$96,426.00	8/1/2016	7/31/2017	Year 1
$94,655.00	8/1/2017	7/31/2018	Year 2
$96,911.00	8/1/2018	7/31/2019	Year 3

Summary Our aim is to develop a therapeutic for myasthenia gravis (MG) which would eliminate the need for corticosteroids, the primary treatment. A central question for MG, and autoimmune disorders in general, is how autoreactive immune cells avoid elimination. We have discovered the presence of an inhibitor of apoptosis protein, survivin, in the thymus and B cells from MG patients. Our hypothesis is that survivin supports the presence of autoreactive immune cells by allowing these cells to escape cell death. By targeting survivin-expressing cells in animal models of MG, we have shown the reduction in acetylcholine receptor antibodies which cause the disease. Our proposal contains two specific aims. The first aim will assess thymus tissue from patients with myasthenia gravis and healthy controls for survivin expression. We will utilize specimens from three centers with large MG populations and the NIH-sponsored MGTX trial. The second aim will evaluate survivin therapeutics in a myasthenia gravis rodent model. The aim will assess the ability for survivin therapeutics to improve observable weakness, decrease the expression of autoreactive immune cells, decrease acetylcholine receptor specific antibodies, and decrease damage to the nerve-muscle junction. Fulfillment of our aims will provide a new fundamental understanding of basic mechanisms of autoimmunity. Successful completion of our studies will validate survivin-targeted approach for therapeutic development.

Maria Chiara Manzini Ph.D.

RG Unraveling the phenotypic variability of alpha-dystroglycanopathies

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

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

will also determine how different mutations affect dystroglycan and how therapeutic strategies may vary depending on the affected enzyme.

FLORIDA

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

Carlos T. Moraes Ph.D.

RG Reducing the levels of mtDNA mutations by mitochondrial nucleases

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

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

Gainesville - University of Florida

Andrew Berglund Ph.D.

RG Stabilization of toxic RNA provides novel insights into myotonic dystrophy

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

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

Rebecca Willcocks Ph.D.

DG MRI, MRS, and functional characterization of the arm in DMD

$56,121.00	8/1/2015	7/31/2016	Year 1
$59,964.00	8/1/2016	7/31/2017	Year 2
$55,337.00	8/1/2017	7/31/2018	Year 3

Summary Boys and men with Duchenne muscular dystrophy (DMD) experience progressive muscle weakness and difficulty with everyday activities. Many drugs that might slow or reduce the symptoms of DMD are in clinical or preclinical trials. These trials often use walking performance as the most important outcome measure, so boys who are not able to walk are excluded from participation. Boys and men with DMD are unable to walk for a significant part of their life, so it is important to examine whether potential therapeutics can help them to maintain their ability to eat and drink, perform personal care, and use technology, and use a wheelchair. In fact, several biotechnology companies are already working to include arm function measurements in clinical trials. Using magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), we can noninvasively examine how DMD affects the muscles. In this project, we will use MRI and MRS to describe how DMD affects the shoulder, upper arm and forearm muscles of 9-18 year old boys with DMD, and how these muscles change over 1 year. We will also examine the relationship between MRI and MRS

measures in the arm muscles and tests of arm function, which will include reaching, grasping, and moving objects. If the MRI and MRS measures are sensitive to the disease and predictive of functional performance, they might be useful to evaluate the impact of drugs in nonambulatory boys and men in clinical trials.

Jacksonville - Mayo Clinic Jacksonville

Tania F Gendron Ph.D.

RG16 Investigating lymphocyte and spinal fluid c9RAN proteins as c9ALS biomarkers

$95,000.00	8/1/2016	7/31/2017	Year 1
$95,000.00	8/1/2017	7/31/2018	Year 2
$95,000.00	8/1/2018	7/31/2019	Year 3

Summary A C9ORF72 mutation is the most common known cause of amyotrophic lateral sclerosis (ALS), a devastating motor neuron disease. This mutation gives rise to RNA believed to wreak havoc in the brain of "c9ALS" patients. For instance, it causes the production of potentially harmful "c9RAN" proteins. Evidence that this RNA is toxic has spurred the field to investigate therapeutic approaches to counteract it. Yet, success in developing an effective treatment will require all aspects of the drug discovery process to be addressed, including the identification of powerful biomarkers to measure a patient's response to treatment and to indicate prognosis and disease stage. We reported that poly(GP), an abundant c9RAN protein in c9ALS, is detected in patient cerebrospinal fluid (CSF), and we recently discovered poly(GP) is present in lymphocytes collected from blood. Since poly(GP) is unique to C9ORF72 mutation carriers, and since therapeutic strategies currently under investigation cause decreases in poly(GP), we postulate poly(GP) will serve as important clinical and pharmacodynamic biomarkers. To investigate this, we will take advantage of CSF and lymphocytes collected longitudinally from c9ALS patients as part of a separate study and determine whether poly(GP) associates with clinical features of disease. Furthermore, we will probe the effectiveness of poly(GP) in predicting treatment response using c9ALS patient lymphoblastoid cell lines and CSF from mice engineered to model c9ALS.

Leonard Petrucelli Ph.D.

RG16 Modeling repeat-specific functions and selective vulnerability in C9orf72-ALS

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease without cure or effective therapeutics. A common cause of this disease is a repeat expansion in the gene C9orf72. This repeat is transcribed into RNA that accumulates into foci throughout the brain and spinal cord. The repeat produces toxic repetitive proteins through a process called RAN translation. And the repeat also leads to the aggregation of a protein called TDP-43 through an unknown mechanism. Interestingly, a similar repeat causes a brain disease called spinocerebellar ataxia type 36 (SCA36). Compared to C9orf72, this repeat also forms foci and some of the same proteins, but does not cause TDP-43 aggregation and affects different neurons. In this study, we aim to compare the effects of expressing the two repeats in mice to determine what cellular functions are affected by the expression of the C9orf72 repeat and not by the SCA36 repeat. We believe these functions will be most important for TDP-43 aggregation and ALS-specific neuronal death. We also wish to explore the effect of expressing the C9orf72 repeat in the cerebellum, a brain region that degenerates in SCA36 but not in ALS. The repeat normally accumulates in this region in patients, and its expression in these neurons may play a role in ALS. Ultimately, if we can understand why neurons respond differently to each repeat, and what induces TDP-43 aggregation, we can use that knowledge to better understand and hopefully treat ALS.

Miami - University of Miami School of Medicine

Antoni Barrientos Ph.D.

RG15 Role of cysteine-rich proteins in mitochondrial cytochrome c oxidase biogenesis

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Defects in mitochondrial cytochrome c oxidase (COX) assembly are a frequent cause of mitochondrial encephalomyopathies in humans. This enzyme, formed by multiple proteins, is necessary for cellular respiration and for cellular energy production. COX does its functions thanks to metal groups that are incorporated into two of its proteins. Copper delivery and insertion into COX require COX-specific metallochaperones, COX11 and SCO1/2 that receive copper from COX17, a cysteine-rich protein. Several additional proteins of the COX17 family exist in mitochondria, whose role/s remain unknown. The main objective of this project is to gain insight into the role of three of these proteins: CMC1, CMC2 and PET191. We will use innovative human cultured cell models knockout for these genes, and cell lines from patients with mutations in PET191 and COX copper chaperones to gain insight into the role of CMC1, CMC2 and PET191 in human mitochondrial COX assembly by: (1) Creating and characterizing the phenotype of gene-specific knockout human cells prepared by using innovative gene editing technology. 2) Identifying the proteins that functionally interact with CMC1, CMC2 or PET191, and 3) Analyzing the cellular and mitochondrial copper levels and the redox environment in the knockout human cell lines as well as in cell lines overexpressing the proteins under study.

GEORGIA

Atlanta - Emory University

Jonathan D. Glass MD

RRG Clinical Research in ALS (CRIALS)

$158,000.00	12/1/2015	11/30/2016	Year 1

Summary Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with wide variability in clinical features. This variability includes age of onset, rate of progression, and degree of disability, leading clinicians and investigators to question whether ALS represents a disease with a number of underlying mechanisms and causes. Our clinical center has expertise in clinical characterization of patients, collection of biospecimens (blood, DNA, spinal fluid, and autopsy tissues), and correlation and databanking of clinical information. These data and biopspecimens are essential building blocks for research into pathogenesis and treatment of ALS. We share these materials with investigators around the world. This proposal is to provide funding for the infrastructure needed to maintain and grow this valuable resource of ALS research.

H. Criss Hartzell Ph.D.

RG Molecular Mechanisms of Muscular Dystrophies Caused by ANO5 Mutations

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2

Summary Recessive mutations in the ANO5 gene cause a spectrum of muscular dystrophies that affect both proximal and distal muscles including limb-girdle muscular dystrophy (LGMD2L) and Miyoshi muscular dystrophy (MMD3). Although a growing number of muscular dystrophy patients have ANO5 mutations (estimated ~25% limb-girdle muscular dystrophies), almost nothing is known about the function of the ANO5 protein or about how its dysfunction causes muscular dystrophy. We have exciting new data that the ANO5 protein is not a chloride ion channel as was previously thought, but rather is a phospholipid scramblase that plays a major role in trafficking and fusion of cell membranes during muscle repair. In healthy individuals, normal exercise produces tears in the plasma membrane that are healed by two processes: (1) resealing of small lesions by assembly of new plasma

membrane to fill the holes and (2) fusion of muscle progenitor stem cells (satellite cells) to regenerate new muscle fibers at sites of more severe damage. Disruption of ANO5 function by disease-causing mutations would impair these intrinsic mechanisms of muscle repair. If ANO5 is a phospholipid scramblase as we hypothesize, and not a chloride channel as previously thought, this opens a completely novel line for development of therapies for muscular dystrophies, especially those caused by ANO5 dysfunction, but potentially also other types of muscular dystrophies caused by muscle membrane fragility or defective repair.

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

RG16	(RIG) Precision medicine in muscular dystrophy: Variants of unknown signficance			
	$100,000.00	8/1/2016	7/31/2017	Year 1
	$100,000.00	8/1/2017	7/31/2018	Year 2
	$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Gene panels, exome and genome sequencing are now widely used in clinical diagnostics for neuromuscular disorders. These DNA based assays result in detection of a large number of variants of unknown clinical significance (VOUS). According to the recent variant interpretation guidelines, several pieces of data including functional evidence are needed to classify the variant of unknown signficance (VOUS) as being pathogenic or not. Transcriptome analysis can be used for functional assessment of these variants to generate evidence for pathogenicity for variant in known and newly identified and therefore help patients participate in clinical trials and drive precision genomic medicine for neuromuscular disoders.

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

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

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

Christine DiDonato PhD

RG16	Deciphering mechanisms underlying muscle dysfunction in SMA mice			
	$100,000.00	8/1/2016	7/31/2017	Year 1

$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary SMA is caused by low levels of the ubiquitously expressed protein, survival motor neuron (SMN). We have developed long-lived SMA mice in which we have specifically increased SMN in motor neurons. These mice have normal functioning motor neurons, but they still have very clear functional deficits, thus unmasking muscle problems caused by low SMN levels in this tissue. This has important long-term implications for SMN-based therapies under current clinical investigation. Here we will characterize these muscle defects, identify altered molecular pathways in skeletal muscle that are responsible for the observed muscle weakness and atrophy and based or future research aimed at enhancing muscle function and preservation.

Chicago - Johns Hopkins University

Gabsang Lee Ph.D., D.V.M.

RG15 Modeling Duchenne muscular dystrophy with hiPSCs and pharmacological rescue

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Duchenne muscular dystrophy (DMD) is one of the most common muscular dystrophies. DMD is caused by mutations DYSTROPHIN and so far over 1,000 different sequence variations in the culprit gene (www.dmd.ml) have been known. Although several rodent, feline and canine models have provided DMD-related data on pathogenesis, the disease progression in the animals is somewhat different from that in human patients. Humanized DMD models carrying patient-specific DYSTROPHIN mutations will be complementary to current animal models of DMD, and one such example is DMD-specific human induced pluripotent stem cells (hiPSCs). Here, we propose to generate patient-specific myoblasts from DMD-specific hiPSC lines, followed by cellular/molecular characterization for better understanding of the pathogenesis, validating pharmacological and genetic intervention in vitro, and modeling microenvironments of DMD lesions receptive for healthy myoblasts. Our 'DMD-in-a-dish' model will be essential for tackling such a devastating muscular dystrophy.

Chicago - Northwestern University - Chicago Campus

Evangelos Kiskinis PhD.

RG15 Defining the overlap of molecular mechanisms of degeneration in genetic ALS

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary We have successfully been using stem cells generated from the skin of ALS patients to make patient-specific human motor neurons and study their disease in the lab. This novel technology has essentially given us access inside the brain and spinal cord of patients. We have shown that motor neurons with mutations in the SOD1 gene exhibit mitochondrial dysfunction and electrical excitability defects. We have also shown that using small molecules that target these pathways we can extend the survival of patient motor neurons. In this study we propose to extend this approach and generate motor neurons from patients that from a wide range of different genetic ALS types. By studying these different motor neurons and comparing how similar they are in terms of the disease mechanisms we can understand how similar or different ALS patients might be. This will be invaluable in our efforts to: a) design drugs that will target specific patients; b) as well as to identify therapeutics that might be broadly relevant to all ALS patients. In the second part of our proposal we will determine whether the irregular manner in which ALS motor neurons fire electrical signals might contribute to their death. This is an important question as work done in our cellular system as well as in human patients has shown that this is a consistent

problem that may significantly contribute to the disease. If we understand why it happens and how motor neurons deal with it we might discover ways of stopping it.

INDIANA

West Lafayette - Purdue University

Shihuan Kuang Ph. D.

RG Targeting hypoxia signaling to improve the efficiency of myoblast transfer

$84,600.00	5/1/2015	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

Nivedita Jerath M.D.

CRTG Driving Ability in Patients with CMT 1A

$76,140.00	7/1/2015	9/30/2016	Year 2

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

Michael Shy M.D.

RG Identification and Treatment of ER Stress in Patients with CMT1

$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.

Michael Shy M.D.

RIG Inherited Neuropathy Consortium

$159,733.00	2/1/2016	1/31/2017	Year 1
$161,796.00	2/1/2017	1/31/2018	Year 2
$157,270.00	2/1/2018	1/31/2019	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). Over seven thousand partiipants are registered in various INC protocols that investigate how different types of CMT progress, develop outcome measures in children and adults to be used in clinical trial development, identify genetic changes that modify the severity of CMT, and identify new genetic causes of CMT. We also are training the next generation of researchers in CMT, developing standards of care for people with CMT, developing clinical trials and linking with National CMT programs throughout the world.

KENTUCKY

Lexington - University of Kentucky Research Foundation

Haining Zhu Ph.D.

RG FUS phosphorylation and its significance in ALS

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease. Several ALS genes have been identified, including two genes encoding RNA processing proteins TDP-43 and fused in sarcoma (FUS). FUS is a ubiquitously expressed RNA-binding protein that is predominantly localized in the nucleus. FUS plays a role in a variety of processes including transcriptional regulation and mRNA splicing. However, little is known regarding how FUS function is regulated. The hypothesis to be tested is that phosphorylation of FUS by CK2 plays a critical role in regulating FUS function and that inhibition of FUS phosphorylation can mitigate its toxicity. We recently found that FUS is phosphorylated by casein kinase 2 (CK2) and identified several potential phosphorylation sites. Co-expression of CK2 exacerbated the locomotive impairment of FUS transgenic flies whereas the CK2 phosphorylation-deficient mutant of FUS reduced toxicity in flies. The results suggest that phosphorylation of FUS by CK2 can directly modulate FUS toxicity. We will determine the effect of inhibiting FUS phosphorylation on FUS toxicity in vivo using fly models. We will determine the exact CK2 phosphorylation site(s) using mass spectrometry and investigate the significance of individual phosphorylation site. This project will not only yield novel mechanistic insights on regulation of FUS function by CK2 phosphorylation, but also determine whether CK2 inhibition can be a new therapeutic avenue.

MAINE

Bar Harbor - The Jackson Laboratory

Robert W. Burgess Ph.D.

RG Gene therapy approaches for Charcot-Marie-Tooth type 2D

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous collection of conditions that result in fatigue and weakness due to degeneration of nerves in the arms and legs. There is currently no treatment for CMT. Using well-validated mouse models of CMT type 2D, caused by dominant mutations in the GARS gene (glycyl tRNA synthetase), we will perform a proof-of-concept gene therapy experiment. We will target the mutant copy of the Gars gene while leaving the normal copy intact, with the anticipation that this will result in an effective treatment for the neuropathy. Positive results from these studies will have important translational implications not only for CMT2D and related peripheral neuropathies, but also for many dominantly inherited neuromuscular diseases, including muscular dystrophies and motor neuron diseases. The successful completion of these studies will enable us to develop gene therapy approaches for human GARS CMT2D variants.

MARYLAND

Baltimore - Johns Hopkins University School of Medicine

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

RG C9orf72 ALS is caused by nuclear-cytoplasmic trafficking dysfunction

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary A mutation of the gene C9orf72mutation is the most common genetic cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), though the underlying disease mechanism is poorly defined. Furthermore, is turns out to be a cery common caus eof apparent sporadic ALS, in spite of not prior family history. This discovery herald a huge change in ALS biology and understanding the nature of the biological defect is essential to pioneer new approaches to ALS and most important develop a therapy that will affect c very substantial subset of ALS. Our past failure in finding ALS therapies can and will be overcome by understanding these molecular sub-forms of ALS. This proposal will set the stage for such discoveries.

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

RRG Robert Packard Center for ALS Research (Wings 2014) (Rothstein, Jeffrey)

$83,350.92	9/1/2015	8/31/2016	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 who 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.

Baltimore - Johns Hopkins University-School of Medicine

Constantin d'Ydewalle Ph.D.

DG LncRNA as therapeutic target for SMA

$60,000.00	8/1/2015	7/31/2016	Year 1
$60,000.00	8/1/2016	7/31/2017	Year 2
$60,000.00	8/1/2017	7/31/2018	Year 3

Summary Spinal muscular atrophy (SMA) is the leading inherited cause of infant mortality and is caused by mutation of the survival motor neuron-1 gene (SMN1), retention of a highly homologous SMN2 gene, and reduced levels of SMN protein. Hence, SMA treatments currently under development aim to increase SMN protein levels. Prior studies and our

preliminary data show that SMN is highly expressed during early gestational stages, but decreases postnatally. This may indicate that specific levels of SMN are temporally required for normal motor neuronal development. It is unknown, however, how SMN expression is regulated over time. Approximately 90% of the human genome is transcribed while only 2% has protein-coding potential. Recently, long non-coding RNAs (lncRNAs) have been shown to be powerful regulators of gene expression. LncRNAs transcriptionally regulate many genes via interactions with the polycomb repressive complex-2 (PRC2). My work focuses on a SMN-associated lncRNA that regulates the expression of SMN during neuronal development and differentiation. The project proposed here aims to understand how this mechanism works in neurons both in mice and in a petri dish. A better understanding of the mechanisms that regulate SMN expression are crucial for SMA therapeutics development.

Thomas Philips Ph.D

DG15 The role of monocarboxylate transporters in motor neuron disease

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary Motor neuron degenerative diseases like amyotrophic lateral sclerosis (ALS) are characterized by the progressive degeneration of the motor neuron, the cells that control voluntary movement by acting on specific muscle subtypes. In ALS patients, degeneration of motor neurons leads to muscle wasting, spasticity, paralysis and death approximately three to five years after disease onset. No cure for this disease is currently available. One of the hallmarks of motor neuron degeneration is the reduced trophic support in terms of energy metabolites provided to the motor neuron by cells that support its function, the glial cells. It has recently been established that one glial cell type, the oligodendrocyte (oligo), is involved in providing motor neurons with energy metabolites. Oligos are ideally suited for this role given their strong intimacy with the motor neuron as these cells entirely engulf the motor neuron axon. Specific oligo transporters, the monocarboxylate transporters, mediate the transfer of energy metabolites from oligo into neurons. As motor neurons can be as long as four feet in humans, motor neurons are a huge burden on the energy supply available in order to maintain normal homeostasis. In this study, we try to obtain a better understanding of the oligo trophic support to the motor neuron and assess whether we can modulate it in order to prevent motor neuron degeneration.

Baltimore - University of Maryland, Baltimore

Aikaterini Kontrogianni-Konstantopoulos Ph.D.

RG Loss of actomyosin regulation in distal arthrogryposis due to mutant MyBP-C slow

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Contraction of skeletal muscle is a highly regulated process, which involves the sliding of thin actin filaments past thick myosin filaments. When this process is compromised, skeletal myopathies arise with symptoms that may vary from mild to severe, resulting in muscle weakness and potentially death. Myosin binding protein-C (MyBP-C) comprises a family of important regulators of muscle contractility, and is expressed in both skeletal and cardiac muscles. Consistent with this, mutations in the genes encoding the cardiac and slow skeletal isoforms have been causally linked with the development of hypertrophic cardiomyopathy and arthrogryposis myopathy, respectively. Our studies will focus on the characterization of the physiological roles of the slow isoform of MyBP-C in skeletal muscle during normalcy, and how these are compromised in severe and lethal forms of arthrogryposis myopathy. The obtained information will provide important mechanistic insights about the molecular mechanisms that regulate contraction in health, and how these are altered during disease.

MASSACHUSETTS

Boston - Children's Hospital Boston

Alan H. Beggs Ph.D.

RG15 Molecular Genetics of Congenital Myopathies

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary The congenital myopathies are a diverse group of inherited neuromuscular conditions that result in skeletal muscle weakness of variable onset and severity. To better understand the causes of these disorders, which are commonly seen in MDA neuromuscular clinics, we are building an extensive registry and biorepository of cases and specimens from patients and their families. Through new genetic methods such as "whole exome sequencing" of patient's DNA, we are completing the identification of disease genes and genetic mutations that has been ongoing since the 1980's. To study the pathophysiology and provide model systems with which to develop therapies, we are developing zebrafish, mouse and dog models of these mutations. The zebrafish models are particularly powerful as they provide us with the opportunity to screen small molecule libraries to identify new drugs to treat these conditions. In this project we will continue and expand this work to focus on the less well-understood congenital myopathies that are often undiagnosed due to ambiguous results on clinical muscle biopsy. Success will lead to a better understanding of the causes of weakness in congenital myopathies, and to the development of new drug therapies to treat these conditions.

Louis Kunkel Ph.D.

RG15 Genome-scale CRISPR-Cas9 knockout screen to identify genetic modifiers of FSHD

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$99,134.00	2/1/2018	1/31/2019	Year 3

Summary FSHD is the most common autosomal dominant form of muscular dystrophy, yet the underlying genetics is not completely solved. We propose to perform a genetic screening experiment to identify the missing genetic link(s) that may hold answers as to why some individuals that harbor FSHD genetics are not clinically affected, and why individuals with the same disease genetics are differently affected. Using the latest in genome-editing technology, we will perform genome-wide modifications that result in loss-of-function mutations across every gene in the human genome, systematically identifying modifier genes that reduce the phenotypic impact of FSHD when inactivated. Our candidate modifier genes will be cross-referenced to a panel of genomic sequencing data from asymptomatic individuals. We will further validate these genes with functional rescue experiments in our zebrafish model of FSHD, and measure their ability to change the molecular disease signature of FSHD patient cells. Ultimately, we seek to exploit the existence of so-called 'cured' or mildly affected human models of FSHD as a resource to aid in our understanding of FSHD disease genetics, as well as pinpoint concrete targets for drug discovery.

Da-Zhi Wang Ph.D.

RG The miR-155-MEF2A axis in muscular dystrophy

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

Summary Defective muscle regeneration and function is associated with neuromuscular diseases, including muscular dystrophies. However, the molecular targets that regulate skeletal muscle development, function and regeneration remain poorly defined. Our lab has previously demonstrated that muscle-specifically expressed miRNAs, including miR-1, miR-

133 and miR-206, modulated muscle cell and satellite cell proliferation, differentiation and muscle regeneration. Most recently, we found that the expression and function of MEF2A, a member of the MEF2 family of myogenic enhancer factors, was regulated by miR-155. Interestingly, the expression level of miR-155 was increased in the skeletal muscle of mdx mice, an animal model for human Deuschenne Muscular Dystrophy. We further showed that overexpression of miR-155 inhibits myoblast differentiation in myoblast cell line. Most importantly, we found that mice with genetic deletion of miR-155 displayed better muscle function and regeneration. The overall goal of this study is to uncover the involvement of the miR-155-MEF2A axis in muscular dystrophy. We design three aims to achieve this goal, using a mouse model of human muscular dystrophy. Together, our studies will define the biological function of miR-155 in muscle function and regeneration. miR-155 could become a novel therapeutic target to treat muscular dystrophy.

Boston - Northeast ALS Consortium

Jonathan D. Glass MD

RIG Northeast ALS Consortium: Infrastructure support

$50,000.00	9/1/2015	8/31/2016	Year 1
$51,500.00	9/1/2016	8/31/2017	Year 2
$53,045.00	9/1/2017	8/31/2018	Year 3

Summary The Northeast ALS Consortium (NEALS) is an international, independent, non-profit group of 117 research sites around the world who collaboratively conduct clinical research in Amyotrophic Lateral Sclerosis (ALS) and other motor neuron diseases. The mission of NEALS is to translate scientific advances into new treatments for people with ALS and motor neuron disease as rapidly as possible. This proposal is for support of the administrative infrastructure necessary for the functioning of NEALS. Administrative funding will be shared equally with a grant from the ALS Association.

Boston - Trustees of Boston University

Mahasweta Girgenrath Ph.D

RG15 Utilizing natural history to identify optimal timeline for combinatorial therapy

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2

Summary Laminin-deficient congenital muscular dystrophy (MDC1A) is the second most prevalent form of congenital muscular dystrophy (CMD). Children with this disease experience profound muscle weakness from a very young age and are never able to walk on their own. They typically die prematurely due to respiratory failure or failure to thrive. There remains no cure or treatment for MDC1A. Treatment with a single drug has been attempted but is only able to partially ameliorate some disease symptoms. This suggests a combinatorial treatment with more than one drug targeting multiple disease drivers may lead to better amelioration of the disease and result in increased life span and improved quality of life. We propose to use what we know about early disease progression in a mouse model of MDC1A to identify an ideal timeframe for dual treatment that will result in achieving maximal attenuation of disease symptoms to bring it closer to clinical trials.

Boston - Trustees of Boston University, B U Medical Campus

Shinichi Takayama Ph.D.

RG Protein aggregate myopathies and molecular chaperones

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary We propose to identify pathogenic mechanisms and potential therapeutic targets for myofibrillar myopathies and related protein aggregate myopathies including inclusion body

myositis (IBM) and LGMD (1D and 1E). These diseases are characterized by accumulation of abnormal protein aggregates and myodegeneration. Mutations in the genes encoding the molecular chaperones BAG3 and CryAB cause myofibrillar myopathies. To understand how these mutations cause disease, we will use cell cultures and skeletal muscle tissue to identify the molecular mechanisms by which the chaperones BAG3 and CryAB function in protein aggregation, protein folding, and protein turnover. Our long-term goal is to determine how molecular chaperone complexes protect muscle structure and to identify therapeutic approaches that can ameliorate pathology due to mutations in chaperones.

Cambridge - Catabasis Pharmaceuticals

Joanne Donovan M.D., Ph.D.

RG A Phase 1/2 Study of CAT-1004 in Pediatric Patients w/ DMD

$225,800.00	12/1/2015	3/30/2017	Year 1

Summary CAT-1004 is an orally administered small molecule targeted to inhibit NF-kB in development for Duchenne Muscular Dystrophy (DMD). In the mdx mouse and Golden Retriever Muscular Dystrophy models treated for 6 months, improvements in muscle function were seen with CAT-1004 or a closely related analog. Toxicology studies did not identify dose limiting toxicities. In Phase 1 studies of up to 14 days in adults, no safety signals were observed. In Phase 1 evaluations, targeting of NF- kB in peripheral blood mononuclear cells was shown. Since magnetic resonance imaging (MRI) (T2) has shown progressive leg muscle degeneration that reverses with steroids, a proof-of-concept study of CAT-1004 with MRI is planned. This two-part Phase 1/2 study will evaluate safety, pharmacokinetic and pharmacodynamics of CAT-1004 in boys aged 4 to 7 with DMD and not on steroids. Baseline data will be collected, including MRI of lower and upper leg muscles, timed function tests (TFT: 10 meter walk/run, time to stand, 4-step climb),and muscle strength. In the first part, safety and PK will be assessed for 7 days at multiple doses (n=6 each). Doses will then be selected for a 12-week double-blind, placebo controlled study (n=18-24). MRI assessments of lower and upper leg muscles will be conducted at 0 and 12 weeks and TFT will be conducted monthly. The purpose of this application is to request funds for travel for patients and their families to central sites (ImagingDMD).

Cambridge - President & Fellows of Harvard College

Natalia Rodriguez Muela Ph.D.

DG15 Autophagy controls SMN protein degradation

$59,995.00	2/1/2016	1/31/2017	Year 1
$59,995.00	2/1/2017	1/31/2018	Year 2
$59,995.00	2/1/2018	1/31/2019	Year 3

Summary Spinal muscular atrophy (SMA) is a motor neuron disease and the leading genetic cause of infant mortality. SMA is caused by mutations or the complete deletion of the SMN gene that lead to the deficiency of the survival motor neuron (SMN) protein. It is unanimously accepted that increasing SMN levels restores the diseased phenotype and will be therapeutically valuable for treating patients with SMA. Accumulating evidence suggests that the ubiquitin/proteasome system regulates SMN protein levels. However, whether the other major catabolic mechanism within the cell, the lysosomal/autophagy pathway, is also involved in SMN degradation is completely unexplored. Autophagy is a highly conserved intracellular degradative pathway that plays a critical role in the removal of cell components to maintain cellular homeostasis and has been implicated in the pathology of many neuromuscular disorders. Whereas the proteasome is in charge of degrading short-lived single proteins, autophagy engulfs protein aggregates, protein complexes and cellular organelles and it can do so in a bulk or selective way. Multiple links demonstrating cross-talk between these degradative systems have been reported. The overall goal of this study is to explore the role that autophagy plays in controlling SMN protein levels. Specifically blocking autophagy-dependent SMN degradation may constitute a new approach to find new targets for SMA therapeutic intervention.

Worcester - University of Massachusetts Medical School

Peter L Jones PhD

RG15 FSHD-like mice for therapeutic development and preclinical testing

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary A major impediment to developing ameliorative treatments for facioscapulohumeral muscular dystrophy (FSHD) is the lack of a viable, robust, and consistent phenotypic FSHD-like animal model. We have addressed this void by generating a novel transgenic mouse model based on the widely accepted model for FSHD being caused by increased expression of the DUX4 gene thereby causing a cascade of events leading to FSHD pathophysiology. Thus, the DUX4 mRNA, DUX4 protein and downstream targets are all potential targets for therapeutic development. We have successfully engineered lines of mice that contain the human DUX4 gene while maintaining its native human gene structure. Initial characterization of this mouse indicates that it is healthy and fertile in the absence of inducing DUX4 expression. Induction of DUX4 causes a muscular dystrophy-like phenotypes ranging from very mild to very severe. One can imagine that different disease courses may have different application for therapeutic testing. Therefore, here we will determine the precise conditions necessary to develop multiple screenable FSHD-like phenotypes in this mouse with varying courses of pathology. In addition, we will characterize the natural history of disease in the models. Successful completion of this project will provide the FSHD field with valuable tools for screening numerous classes of potential FSHD therapeutics aimed at DUX4 and its downstream targets and ultimately lead to ameliorative treatments for FSHD.

MICHIGAN

Ann Arbor - The Regents of the University of Michigan

Anthony Antonellis Ph.D.

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

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

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

Asim Beg Ph.D.

RG15 The Rac-GAP a2-chimaerin: a new target for motor neuron protection in ALS

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is mostly a sporadic disease, but does have genetic origins. Regardless of cause, motor neuron degeneration is the unifying feature leading to paralysis and death. There are no treatments that halt disease onset or progression, highlighting the urgent need for therapies that positively modify disease course. Dismantling of neuromuscular junctions (NMJ), the site where a motor neuron axon communicates with a muscle cell, is an early pathological event that precedes motor neuron death. High expression of the repulsive axon guidance receptor EphA4 can initiate axonal retraction and NMJ degeneration in human ALS patients and animal models. Inhibiting proteins that relay destructive EphA4 signals is an attractive strategy to prevent these pathogenic events. We show that blocking a2-chimaerin, a critical EphA4 interacting protein, protects motor neurons from degeneration, delays disease onset and extends lifespan in ALS animal models. We hypothesize these beneficial effects are due to increased cytoskeletal health which prevents axonal retraction and NMJ degeneration. We will determine the precise cellular and molecular mechanisms underlying a2-chimaerin-dependent neuroprotection. Our findings will provide a first step toward decoding the molecular logic of EphA4-dependent motor neuron degeneration. This work will provide new insights into disease pathogenesis and may lead to new and more specific therapeutic strategies for treating ALS.

MINNESOTA

Minneapolis - Regents of the University of Minnesota - Twin Cities

James M. Ervasti Ph.D.

RG	Non-invasive biomarkers of defective mitochondrial metabolism in DMD			
	$100,000.00	8/1/2015	7/31/2016	Year 1
	$100,000.00	8/1/2016	7/31/2017	Year 2
	$100,000.00	8/1/2017	7/31/2018	Year 3

Summary While several experimental therapies are in development for Duchenne muscular dystrophy (DMD), a significant obstacle for conducting clinical trials has been the lack of a simple and reliable method to measure therapeutic efficacy. To search for a molecular biomarker capable of monitoring disease progression, we measured the concentration of several hundred metabolites in urine from the mdx mouse model of DMD. Intriguingly, the concentration of Krebs cycle metabolites was significantly lower in the mdx mice as well as a small cohort of DMD patients. Since the Krebs cycle is necessary for producing energy in the form of ATP, our observation synergizes with previous work describing a global "energy-crisis" as a component of DMD pathology. We also have evidence that reduced Krebs cycle capacity may be an important mechanistic component of the skeletal muscle pathology associated with DMD. The goal of our project is to elucidate the role of Krebs cycle dysfunction in skeletal muscle pathology and to measure these metabolic changes in a large cohort of DMD patients in an effort to develop a non-invasive means of monitoring disease progression, which would improve our ability to conduct clinical trials for new DMD therapies.

Michael Kyba PhD

RG	Determinants of self-renewal and differentiation of satellite cells			
	$100,000.00	8/1/2015	7/31/2016	Year 1
	$100,000.00	8/1/2016	7/31/2017	Year 2
	$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Skeletal muscle is highly regenerative, thanks to a population of satellite cells, stem cells for skeletal muscle that differentiate into muscle fibers when necessary and self-renew for the lifetime of an organism. Our understanding of how satellite cells undertake decisions to proliferate, self-renew or differentiate is very limited. This is important because the ability to regenerate shows wide variation between individuals, and declines precipitously as muscular dystrophy advances. This proposal is focused on discovering and studying genes

that regulate the regenerative potential of satellite cells, and will lead to a better understanding of skeletal muscle regeneration, with implications for our understanding of skeletal muscle regeneration in disease states, and of the differences in muscle regenerative potential between individuals.

DeWayne Townsend D.V.M., Ph.D.

RG Hypoxia as a Modulator of Dystrophic Cardiomyopathy

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Duchenne muscular dystrophy (DMD) is a fatal disease resulting from a combination of respiratory and cardiac failure. Despite the importance of these two critical physiological systems, very little is known regarding how they may interact during the progression of DMD. We propose that decreases in oxygen, secondary to respiratory dysfunction, are particularly injurious to the dystrophic heart. The central hypothesis is that dystrophic muscle cells use oxygen less efficiently and have difficulty generating energy at low oxygen levels. We have shown that dystrophic mice subjected to reductions in oxygen develop a metabolic acidosis. This is a condition where the tissues of the body produce excess acid and is most likely associated with shifts to anaerobic metabolism (energy production without using oxygen). However, in highly metabolic tissues, such as the heart, anaerobic metabolism is unable to meet the energy demands. These energy starved cells are more susceptible to damage following even normally tolerated stresses. The presence of a significant role for hypoxia in the progression of dystrophic cardiomyopathy would have a direct impact on the decisions regarding when to initiate ventilatory support. The studies proposed here will assess the importance of hypoxia using both mouse models and experiments in DMD patients. It is hoped that by moving directly into the patient population that we can expedite our understanding of the role of hypoxia in the pathophysiology of DMD.

MISSOURI

Columbia - The Curators of the University of Missouri

Christian Lorson PhD

RG15 Utilizing E1-targeting ASO Morpholinos in a combinatorial strategy for SMA

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Spinal Muscular Atrophy (SMA) is the leading genetic cause of infantile death yet there currently is no effective treatment. The goal of this project is to deliver an optimized anti-sense oligonucleotide in combination with factors that address distinct functional pathways that are deficient in SMA. This work could provide evidence for a new combinatorial approach to SMA that would address a broad range of patient needs.

St. Louis - Washington University in St.Louis

Anne M Connolly M.D.

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

$.00	1/1/2016	3/1/2017	Year 4

Summary While it has been known for many years that corticosteroid use benefits boys with DMD, most clinicians do not consider treating until after age 3 or 4 years of age. The primary reason for the delay is that daily corticosteroid use has many side effects including short stature, obesity, and osteoporosis. A recent randomized blinded study of weekend oral corticosteroid use over one year showed equal improvement in strength with fewer side effects, particularly as related to growth and cushingoid changes. We will test the efficacy of

oral weekend corticosteroid use in infants and young boys with DMD who are under age 30 months. We have demonstrated that the Bayley-III Scales of Infant development shows that infants and young boys in this age group who are untreated decline in abilities when compared to their peers. Furthermore, the North Star Ambulatory Assessment which scores the ability to walk, run, and take steps shows scores that are lower than typically developing boys. Here, in this Phase 2 historically controlled trial, we will use these two measures and treat boys at five MDA-DMD centers.

Aaron DiAntonio M.D.,Ph.D.

RG Mechanism of axon loss in congenital motor neuropathies

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary The nervous system controls muscle contraction via signals sent from motor neurons in the spinal cord to muscles throughout the body. These signals travel down axons, which are long, thin processes connecting the cell bodies of neurons to muscles. In peripheral neuropathies, damage or loss of these axons disrupts neural control of muscle contraction and can severely impair neuromuscular function. We wish to understand how damaged axons degenerate, in order to identify new therapies that will protect axons in patients with peripheral neuropathy. We have identified the protein Sarm1 as an essential component of the axonal degeneration program. It is necessary and sufficient to induce the degeneration of injured axons. Furthermore, Sarm1 function is evolutionarily conserved—it promotes axonal degeneration in both fruit flies and mice and, hence, likely serves a similar function in people. We will use powerful genetic tools available in the fruit fly to identify the genes that act in response to Sarm1 activation. We will search for genes that when inhibited can block the destructive consequences of Sarm1 activation and maintain healthy axons. Once we identify these genes, we will test whether they have a similar function in mammalian neurons. These genes and the proteins made by these genes will be new therapeutic targets whose inhibition could block axon degeneration and maintain healthy connections between motor neurons and muscles in peripheral neuropathies.

Kelly Renee Monk Ph.D.

RG Control of myelination by G protein-coupled receptor signaling

$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.

Daniel Summers Ph.D

DG Molecular Mechanisms of NAD+ Homeostasis in Peripheral Neurons

$60,000.00	8/1/2015	7/31/2016	Year 1
$60,000.00	8/1/2016	7/31/2017	Year 2
$60,000.00	8/1/2017	7/31/2018	Year 3

Summary Neuromuscular disorders such as Charcot-Marie Tooth diseases are caused by poor communication between muscles and the neurons in the peripheral nervous system that control muscle function. In these diseases, genes are activated that turn on a self-destructive program that eventually degrades neuron function and contact with the muscle. Identifying the genes responsible for neuronal degeneration and how they work will lead to new therapeutics. I am investigating how a gene called Sarm1 contributes to neuron destruction. Activating Sarm1 leads to the loss of a metabolite called NAD+ that is absolutely essential for neuron health and survival. My studies will address how Sarm1 degrades this metabolite and affects neuron health in disease models of Charcot-Marie Tooth disorders. The goal of my work is to identify new targets for therapeutic intervention in these devastating disorders.

Conrad Chris Weihl M.D., PhD.

RG16 Therapeutic modulation of chaperone function in LGMD1D

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Protein aggregates are present in age-associated degenerative disease, including debilitating myopathies and muscular dystrophies. They form when proteins misfold, self-assemble and elude degradation. Protein chaperones, or heat shock proteins (HSPs), protect against the toxic misfolding and aggregation of proteins. Hence, mutations or deficiencies in the chaperone network lead to disease. Recently, we found that DNAJB6, an HSP40 co-chaperone, is mutated in a dominantly inherited inclusion body myopathy (IBM) also named limb-girdle muscular dystrophy type 1D (LGMD1D) (1). LGMD1D is a progressive late onset muscular dystrophy. This proposal will understand the role of DNAJB6 mutants in a degenerative myopathy. The goals of this proposal are to explore novel therapeutics for LGMD1D.

NEW JERSEY

Bridgewater - SANOFI-AVENTIS U.S. INC

Paul August Ph.D.

RG15 ALS Patient Derived Neuron-Muscle Contraction Unit on a Chip

$99,500.00	2/1/2016	1/31/2017	Year 1
$99,500.00	2/1/2017	1/31/2018	Year 2
$99,500.00	2/1/2018	1/31/2019	Year 3

Summary Neurons are a major part of the electrical system in the human body and skeletal muscles are the main contraction units that are activated by neuronal stimulation. In order to fully model how neuronal function is altered in disease states, the neurons need to be connected to muscles that respond to their stimulation. Historically, animal models have not represented neuromuscular diseases very well which has created difficulties for advancing new therapies. We propose to create a human motor neuron and skeletal muscle contraction unit in the laboratory to more appropriately model human neuromuscular disease, the neuromuscular junction and to permit the rapid evaluation of therapeutic pharmacological agents in the laboratory. This system would be designed entirely using human derived cells from patients with ALS disease.

Christopher Penton Ph.D.

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

$60,000.00	6/1/2015	10/31/2016	Year 3

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

Newark - Rutgers, The State University of New Jersey-RBHS-NJMS

Diego Fraidenraich Ph.D.

RG16 Aberrant connexin-43 production in muscular dystrophy

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary In muscular dystrophy it is not well understood why certain fibers are more susceptible to damage than others, or how necrosis spreads to neighboring fibers. Our long-term objective is to investigate the mechanisms of cell-cell communication leading to the expansion of damage in DMD. To achieve this goal, we have studied mdx mice with no dystrophin. We have also injected muscular dystrophy (mdx) embryonic stem cells (ESCs) into wild-type (WT) blastocysts to generate mdx/WT chimeras with reduced dosage of dystrophin. We have recently shown that Cx43, which communicates apposing cardiomyocytes via gap junction channels, plays a critical role in the onset of arrhythmias and lethality of muscular dystrophy mice under stress (Gonzalez et al., Nature Sci Rep, 2015). In preliminary studies for this proposal, we show that connexin 43 (Cx43) is ectopically expressed in skeletal muscle fibers in DMD mouse and human. Because denervated fibers express Cx43 before becoming apoptotic/necrotic, we believe that the aberrant expression of Cx43 in dystrophic muscles potentiates the appearance of necrotic foci. In this study, we will determine whether genetic reduction in the Cx43 dose in mdx:Cx43(+/-) mice and in mdx/Cx43(+/-) chimeras attenuates the extent of necrotic damage and ameliorates disease. Because Cx43 is being considered as a target for important cardiac pathologies, the accomplishment of this proposal will help to elucidate unsuspected new roles for connexins in skeletal muscle.

NEW YORK

Bronx - Albert Einstein College of Medicine, Inc.

Morayma Reyes Gil M.D., Ph.D.

RG Role of PDGF Receptor alpha signaling in DMD cardiac fibrosis

$100,000.00	8/1/2015	1/31/2017	Year 3

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

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

Elisabetta Babetto Ph.D.

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

$50,760.00	5/1/2015	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.

New York - Columbia University Medical Center

Hiroshi Mitsumoto MD

RRG	2014 Wings Over Wall Street Research Projects Proposal			
	$83,350.92	3/1/2016	2/28/2017	Year 1

Summary The MDA Wings Over Wall Street funding (Wings) has extensively supported many projects directly and indirectly at Columbia's MDA/ALS Research Center. For example, we have published approximately 130 papers in refereed journals since the inception of Wings in 2001. During this period, we obtained four large multicenter NIH grants (two clinical trials and two observational studies), two CDC/ATSDR grants, three NIH conference grants, and several MDA research grants. We also organized two large international conferences in 2004 and 2011, and we will hold another large, international Clinical Trials Guidelines Workshop in 2016. This year, we are asking Wings to supplement the last phase of the ALS COSMOS study to complete statistical analyses and to support the ARREST-control project. Wings' support has helped us greatly in generating highly productive research at the MDA/ALS Center at Columbia University.

Livio Pellizzoni Ph.D.

RG15	Role of defective U7 snRNP biogenesis in spinal muscular atrophy			
	$100,000.00	2/1/2016	1/31/2017	Year 1
	$100,000.00	2/1/2017	1/31/2018	Year 2

Summary Spinal muscular atrophy (SMA) is a motor neuron disease caused by ubiquitous deficiency in the SMN protein. SMA is the most common genetic cause of infant mortality and no effective treatment is currently available. Although several strategies to restore SMN levels have shown promising results in preclinical studies and are currently being tested in clinical trials, it remains essential to understand the still elusive mechanisms by which low SMN causes SMA and to define other therapeutic targets that can be pursued in parallel to SMN-enhancing approaches. To address these issues, this project will determine whether disruption of a novel function of SMN in RNA regulation—which we have identified for its potential involvement in the disease process—contributes to SMA pathology in a well-established mouse model that recapitulates many features of the human disorder. Collectively, our studies are designed to elucidate the molecular mechanisms of SMA and have the potential to identify new research avenues for the development of therapeutic approaches for this incurable disease that could complement current strategies for upregulation of SMN.

Liza Pon Ph.D.

RG A newly identified congenital muscular dystrophy: mechanisms and interventions

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,002.00	8/1/2017	7/31/2018	Year 3

Summary Congenital muscular dystrophy (CMD), one of the most frequent dystrophies of childhood, is characterized by neonatal muscle hypotonia, muscle weakness, stiff or frozen joints and delayed motor milestones. The focal point of these studies is CHKB CMD, a newly identified CMD in which patients exhibit generalized muscle wasting and weakness from early infancy with ambulatory delays and severe mental retardation. CHKB CMD patients can die as early as 2 years of age from cardiomyopathy. Currently, there is no cure for CHKB CMD. We will study the mechanism underlying CHKB CMD and possible therapeutic interventions.

Catarina M. Quinzii M.D.

RG Investigating the Pathogenesis of Encephaloneuromyopathy due to RMND1 mutations

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

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

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

Marilena D'Aurelio Ph.D.

RG16 Intermediary metabolism biomarkers in mitochondrial myopathies

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary Mitochondrial diseases are heterogeneous genetic disorders caused by impairment of the system producing energy in mitochondria and manifest with severe myopathic and neurological features. Although the genetic defects are known, many aspects of the disease pathogenesis are yet to be elucidated. Amino acid metabolism is connected with glucose metabolism and the mitochondrial energy-generating system through the tricarboxylic acid (TCA) cycle. In muscle of patients with severe mitochondrial myopathy associated with Myoclonus Epilepsy and Ragged Red Fibers (MERRF) we find increased muscle protein breakdown and increased glutamate and alanine. Our hypothesis is that in mitochondrial disorders, increased utilization of glutamate into the TCA may affect amino acid metabolism and alter the homeostasis of essential metabolites in vital organs thus contributing to the pathogenesis of mitochondrial diseases. Interestingly, in Autosomal Dominant Optic Atrophy (ADOA) patients, a disease with milder myopathy, alanine is not increased, suggesting that specific metabolites, products of the amino acids metabolism could be used as biomarkers in mitochondrial diseases. We propose to quantify metabolites of 80 muscles and 30 sera

from patients with different mitochondrial myopathies and controls. We will uncover altered pathway of the amino acid metabolism to be targeted by therapeutic metabolic supplementation and prognostic biomarkers for monitoring disease progression.

Giovanni Manfredi M.D., Ph.D.

RG15 CHCHD10 in familial ALS

$99,999.00	2/1/2016	1/31/2017	Year 1
$99,999.00	2/1/2017	1/31/2018	Year 2
$99,999.00	2/1/2018	1/31/2019	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a rapidly fatal neuromuscular degenerative disorder, affecting the motor neurons. It leads to paralysis and death in a short period of time and to date there are no cure or effective treatment available. There are several different genetic forms of ALS and the gene mutations responsible for the disease have been identified in almost 80% of the familial cases. Mitochondria are organelles crucial for energy metabolism in all cells, but neurons are particularly susceptible to mitochondrial dysfunction. Many lines of evidence point to mitochondria as targets of ALS disease mechanisms and very recently a new gene has been identified as causative of familial ALS, which encodes for a mitochondrial protein, CHCHD10. The function of the protein is unknown and the mechanisms of disease remain to be elucidated. Therefore, this application will take on the task of understanding how the protein works in mitochondria and how mutations cause neurodegeneration, using novel cellular and mouse disease models. The goal is to shed new light on the causes of ALS and specifically the involvement of mitochondria.

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

Howard J. Worman M.D.

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

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

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

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

James Manley Ph.D

RG15 Senataxin, mutated in ALS4, regulates autophagy

$99,528.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$99,917.00	2/1/2018	1/31/2019	Year 3

Summary Neurological diseases are disorders of the brain, spinal cord and nerves that control the body. Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease and is caused by degeneration of motor neurons in the brain and spinal cord. The disease progression is usually extremely fast and ALS patients die in a course of 3 to 5 years after being diagnosed. We study a specific and unusual type of familial ALS, called ALS4. ALS4 begins in childhood or adolescence and has a slow rate of progression and does

not affect patient's life span. However, like other ALS patients ALS4 patients suffer from progressive weakness of the limbs and face severe disabilities. Mutations in the gene encoding the protein Senataxin (SETX) are responsible for ALS4. Importantly, we found that the absence of SETX leads to a defect in the clearance of unwanted and defective components of the cells (a process called autophagy), what can lead to cell toxicity and ultimately cell death. Defects in autophagy have been reported in numerous neurological disorders that include Alzheimer's and Parkinson's diseases and ALS. Our studies are aimed at dissecting how SETX participates in autophagy regulation and importantly, how ALS4 mutations can affect this process. A fuller understanding of the molecular function of SETX in regulation of autophagy will lead to a better understanding of ALS, and ultimately to novel therapeutic approaches to prevent and treat the disease.

Rochester - University of Rochester

Robert Griggs M.D.

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

$85,680.00	10/1/2015	9/30/2016	Year 3

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

Charles Thornton MD

CRNG Myotonic Dystrophy Clinical Research Network

$306,000.00	1/1/2016	12/31/2016	Year 1
$306,000.00	1/1/2017	12/31/2017	Year 2
$306,000.00	1/1/2018	12/31/2018	Year 3

Summary The goal of this project is to support a Clinical Research Network for studies of myotonic dystrophy. Five centers are participating 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 optimize the methods for testing of new treatments. Another goal is toe understand genetic factors that explain the tremendous variability of how people are affected by myotonic dystrophy. The researchers in the Network will work together to standardize the methods for evaluating myotonic dystrophy, and determine the best ways to assess whether new medications are having a beneficial effect.

NORTH CAROLINA

Charlotte - Carolinas Healthcare Foundation

Amy D Harper MD

RRG Natural History and Preclinical Efficacy for AAV-Mediated Gene Therapy in LG2I

$247,387.00	12/1/2015	11/30/2016	Year 1

Summary Natural History and Preclinical Efficacy for AAV-mediated Gene Therapy in Limb Girdle 2I PI: Amy Harper, MD and CoPI: Qi Long Lu, Carolinas Healthcare System Mutations in FKRP gene are associated with a wide range of muscular dystrophies from mild limb-girdle muscular dystrophy (LGMD) 2I to severe Walker-Warburg syndrome (WWS) and muscle-eye-brain disease (MEB). Currently there is no effective treatment available for FKRP-related diseases. Adeno-associated virus (AAV) mediated gene therapy is one of the most promising and fundamental experimental therapies with the possibility to eventually cure the disease. We have been able to identify a vector system that restores function with good gene expression. In this proposal, we will examine the vector system with 2 doses to determine long term effects and potential side effects. The study will also explore the potential toxicity of FKRP overexpression. These effects will be assessed in the FKRP mutant mouse. Our longer-term goal is to apply the selected and tested AAV vector system for clinic trials treating FKRP-related muscular dystrophies.

OHIO

Columbus - The Ohio State University (OSU)

Arthur H.M. Burghes Ph.D.

RG Defining the information for precise genotype-phenotype correlation in SMA

$88,613.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2

Summary The severity of spinal muscular atrophy is determined by the number of intact SMN2 genes. Although assays have been developed that determine SMN2 copy number they do not indicate whether these genes are intact and can modify. We will develop assays that can determine the number of intact copies and improve prediction of SMA severity.

Columbus - The Ohio State University Research Foundation

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

HCTG Motor Function Test Reliability in NeuroNEXT Infant SMA Biomarker Study

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

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

Columbus - The Research Institute at Nationwide Children's Hospital

Kathrin Christine Meyer PhD

DG15 Patient skin derived cells as a potential tool to subgroup ALS diversity

$60,000.00	2/1/2016	1/31/2017	Year 1

$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary The large variability of the disease course and progression in Amyotrophic Lateral Sclerosis (ALS) causes major problems in the understanding of disease mechanisms, as well as the design of clinical trials and the development of new therapeutics. Therefore, improved methods to sub-classify patients based on disease course or reactivity to potential therapeutics are urgently needed. The "direct conversion" is a new technique that allows fast and efficient generation of central nervous system (CNS) cell types involved in the ALS disease mechanism from skin of patients. In this project, we will evaluate whether these cells can be used for sub-classifying patients based on how they affect motoneurons that come in contact with them. Further, we can test how these patient cells react to different drugs or disease modifyers. Our preliminary data suggests that these patient cells display variable reactions to treatment. In this study, we will discern the molecular differences between patient derived CNS cells that either have a mild or severe effect on motoneuron death. Our project should help to find disease modifiers and novel potential therapeutic targets. In addition, we will evaluate the abundance of misfolded SOD1 in a large number patients that do not carry mutations in this protein. This will help to determine whether therapeutics targeting this protein would be suitable for a larger patient population.

Dayton - Wright State University

Mark Rich M.D., Ph.D.

RG15 Developing Therapy for Myotonia Congenita

$82,603.00	2/1/2016	1/31/2017	Year 1
$83,842.00	2/1/2017	1/31/2018	Year 2
$85,120.00	2/1/2018	1/31/2019	Year 3

Summary Myotonia congenita is an inherited muscle disease in which muscle is stiff because it contracts too much. The cause of stiffness is a genetic change in a protein that is involved in electrical signaling. Patients also have weakness when they begin to exercise. This weakness has never been understood. We have discovered the cause of the weakness to be a change in the electrical charge of the muscle fiber. This discovery suggests that in order to treat myotonia congenita we will need to fix both the stiffness and the weakness. While trying to figure out the cause of weakness we have discovered a novel electrical current in skeletal muscle. Our discovery has opened the door to development of new therapy that could greatly improve muscle function in patients. The goal of this proposal is to optimize therapy in the mouse model of myotonia congenita using newly available FDA approved medications, currently used for other conditions. Our results in mice will guide development a clinical trial in patients and hopefully lead to dramatic advances in treatment of myotonia congenita.

OKLAHOMA

Oklahoma City - Board of Regents of the University of Oklahoma, Health Sciences Center

Sanjay Bidichandani PhD

RG Epigenetic Silencing in Friedreich Ataxia

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary People with Friedreich ataxia, the most common inherited ataxia, have an abnormally expanded GAA repeat sequence in both copies of their FXN gene. This leads to altered packaging of the FXN genes that turns off gene expression, and causes a deficiency of the essential protein frataxin. There is currently no approved therapy that can slow or stop the progression of disease. Our experiments are designed to determine the precise mechanism

of this altered packaging in Friedreich ataxia, and to identify therapies to reverse it and restore normal FXN gene function.

PENNSYLVANIA

Philadelphia - Thomas Jefferson University

Angelo C Lepore Ph.D.

RG Toward therapeutic intervention in ALS: role of ephrin signaling in astrocytes

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary ALS is a devastating condition characterized by motor neuron (MN) loss in brain and spinal cord. Nevertheless, studies in ALS animal models and with patient tissues suggest that cellular abnormalities are not limited to MNs. In particular, astrocytes play a key role in ALS progression. However, the astrocyte mechanisms involved in ALS pathogenesis remain largely not understood, hampering development of effective therapies for targeting this cell population and for treating disease. The Eph and ephrin family of molecules plays a number of critical roles in the CNS. A recent finding demonstrated that expression of the Eph receptor, EphA4, in MNs significantly contributes to MN degeneration and overall disease pathogenesis in several ALS animal models and in human disease. EphA4 can be stimulated by binding to ephrin-B ligands, including ephrin-B2. We find pronounced ephrin-B2 up-regulation selectively in astrocytes in areas of MN loss in human ALS spinal cord and in SOD1-G93A mice, the most widely studied ALS animal model. Excitingly, we also find that reducing ephrin-B2 in spinal cord astrocytes prolongs disease in SOD1-G93A mice. These findings suggest that ephrin-B2 is (1) the ligand for pathogenic actions of EphA4, (2) a signaling mechanism underlying astrocyte pathogenicity in ALS, and (3) a promising treatment target. In this project, we will test the hypothesis that abnormal expression of ephrin-B2 in spinal cord astrocytes is a pathogenic mechanism in ALS.

Diane E. Merry Ph.D.

RG Targeting AR toxicity in SBMA through SIRT1 activation

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary Many neurodegenerative diseases result from protein misfolding and accumulation due to a variety of genetic or environmental causes. Spinal and bulbar muscular atrophy (SBMA) is one such disease; it is an inherited, adult-onset, neuromuscular disease that is caused by the expansion of a polyglutamine tract within the androgen receptor (AR) and is related mechanistically to other neurodegenerative diseases caused by polyglutamine expansion. An important feature of SBMA is that its onset and progression are dependent on androgen binding by the mutant receptor. Our studies of mouse and cell models of SBMA that reproduce the androgen- and polyglutamine-dependent nuclear AR aggregation seen in patients, as well as its toxicity, revealed that the mutant AR must be modified by the addition of acetyl groups for its aggregation and toxicity. Moreover, the deacetylase SIRT1 is strongly neuroprotective in cell models of SBMA and this neuroprotection largely depends upon its ability to deacetylate the mutant AR. Here we will investigate the therapeutic potential of activating SIRT1 with small molecule activators. We will build on our preliminary findings that one of the compounds tested to date leads to decreased AR acetylation concomitant with decreased mutant AR aggregation and DHT-dependent toxicity. We anticipate that these studies will reveal new and powerful opportunities for therapeutic development in SBMA.

Davide Trotti Ph.D.

RG Molecular mechanisms of toxicity of the ALS/FTD-linked C9ORF72 gene.

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary The discovery of aberrantly expanded repeat sequences in C9ORF72 gene is of great importance for the field of ALS research because it accounts for a larger proportion of familial and sporadic ALS cases than SOD1. This discovery therefore represents an opportunity to develop different and more impactful therapeutic approaches. Nevertheless, we lack knowledge of both the normal function of the C9ORF72 protein and the potential toxic effect of its aberrant mutations. Our recent work revealed that C9ORF72 neurotoxicity could be due to accumulation of aberrant C9ORF72-derived dipeptides. We recently found that a particular class of these proteins, the arginine-rich dipeptides, are potently toxic to motor and cortical neurons, that is the cell types that are affected in ALS/FTD. Another class of protein dipeptides generated from the C9ORF72 mutated gene, the glycine-alanine dipeptides, also triggered disease-relevant phenotypic changes in neurons, although they did not manifest short term intrinsic toxicity. More importantly, these aberrant proteins were reported in human-induced motor neurons and postmortem spinal cord tissues obtained from C9ORF72 ALS and ALS/FTD patient autopsies, suggestive of their relevance to the human disease. In this study, we propose to investigate the mechanisms by which Proline-arginine exert their neurotoxicity and how the glycine-alanine proteins trigger dysfunction in neurons.

Pittsburgh - University of Pittsburgh

Paula Clemens M.D.

RG15 CINRG Becker Natural History Study - Travel Funding

$8,470.00	11/1/2015	10/31/2016	Year 1
$8,470.00	11/1/2016	10/31/2017	Year 2
$8,470.00	11/1/2017	10/31/2018	Year 3

Summary This proposal is to assist with travel and lodging costs for individuals with Becker Muscular Dystrophy participating in the ongoing study Becker Muscular Dystrophy – A Natural History Study to Predict Efficacy of Exon Skipping.

Stephen Meriney Ph.D.

RG A novel calcium channel agonist as a treatment for LEMS

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

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

TENNESSEE

Memphis - St. Jude Children's Research Hospital

Nam Chul Kim Ph.D.

DG15 Role of a ubiquitin ligase, UBE4B, in mutant VCP-mediated diseases

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary Mutations in Valosin-containing protein (VCP) underlie familial forms of amyotrophic lateral sclerosis and inclusion body myopathy. VCP functions as a ubiquitin-dependent segregase that extracts ubiquitinated targets from a complex structure, thus regulating many distinct cellular processes. The mechanism whereby mutations in VCP cause disease is unknown, no cure or effective treatment exists for patients. In order to elucidate the disease causing mechanism and to identify putative therapeutic targets, we have generated a Drosophila model with mutant dVCP and performed genetic screening. Interestingly, we found the ubiquitin E3/E4 ligase, UBE4B, as a strong genetic modifier. RNAi knock-down of UBE4B strongly rescues degeneration of eyes and neurons caused by VCP mutation. We also have found that UBE4B binds more strongly to mutant VCP. Therefore, I hypothesize that mutant VCP's toxicity is predominantly mediated through increased interaction with UBE4B and its ligase activity and that modulating this interaction may be a beneficial treatment strategy. In this project, I will confirm the results from Drosophila in a mammalian system and test whether inhibiting the enhanced interaction between UBE4B and mutant VCP could be a useful therapeutic strategy. I will identify common interactors and cellular pathways of VCP and UBE4B via interactome analysis by mass spectrometry.

TEXAS

Dallas - UT Southwestern Medical Center

Douglas Anderson Ph.D.

DG15 Mitigating Muscular Dystrophy with a Calcium-regulatory Micropeptide

$60,000.00	2/1/2016	1/31/2017	Year 1
$60,000.00	2/1/2017	1/31/2018	Year 2
$60,000.00	2/1/2018	1/31/2019	Year 3

Summary Defects in calcium handling are a hallmark of muscle diseases, including muscular dystrophies, that activate a series of pathological events that contribute to muscle weakness and damage. In contrast, restoring the normal movement of calcium can ameliorate muscle pathology and improve the performance of damaged muscles. Effective approaches to enhance calcium handling as a treatment for muscular dystrophies are currently unavailable, in part due to a lack of understanding about the molecular mechanisms that regulate calcium handling in skeletal muscle. We recently discovered a novel small membrane protein, called Myoregulin, that functions as a direct inhibitor of the calcium pump that controls muscle contractility. Genetic studies in mice have shown that Myoregulin is an important regulator of muscle performance, however, its role in mediating muscle disease is unknown. Recent preliminary studies have demonstrated that overexpression of Myoregulin alone is sufficient to induce a muscular dystrophy-like phenotype. This proposal aims to characterize the role of Myoregulin as a driver of muscle disease and as a potential therapeutic target for enhancing intracellular calcium handling as a treatment for muscular dystrophies.

Ronald Haller M.D.

RG Impaired Oxidative Capacity in McArdle Disease: Causes and Treatment.

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary This study will investigate the cause of impaired muscle oxidative metabolism when muscle glycogen metabolism is blocked in McArdle disease and will determine the ability of trieptanoin to correct the oxidative defect.

Houston - Baylor College of Medicine

Susan Hamilton Ph.D.

RG15 Molecular Mechanisms and New Interventions for Central Core DIsease

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Central Core Disease (CCD) is associated with mutations in the gene for the skeletal muscle Ca2+ release channel, RyR1. Currently there are no treatments for CCD. RyR1 is an extremely large protein and mutations in different locations within RyR1 have very different functional outcomes. Using two mouse models of CCD (Y522S and I4898T mutations), we show that while both mutations produce the muscle weakness characteristic of CCD, the mechanisms that lead to the muscle weakness are very different. We propose that therapeutic interventions for these two types of CCD will also need to be different. To test this hypothesis, we will test the efficacy of three interventions (identified in our laboratory as having the potential to treat CCD) in the two CCD mouse models to determine if different interventions work better in one type of CCD mouse than in the other. Since the three drugs are already either approved for use in humans or currently in clinical trials for other diseases, these studies will lay the ground work for rapid development of therapeutic interventions for CCD.

KE MA MD, PhD

RG15 The Therapeutic Potential of Circadian Clock Modulators in Muscular Dystrophies

$100,000.00	2/1/2016	1/31/2017	Year 1
$100,000.00	2/1/2017	1/31/2018	Year 2
$100,000.00	2/1/2018	1/31/2019	Year 3

Summary Duchene Muscular Dystrophy (DMD) is characterized by progressive muscle wasting and functional decline due to repeated cycles of degeneration and regeneration. Effective means to maintain critical muscle mass ameliorates muscle dystrophy and greatly improve survival of patients with DMD. Recently studies have demonstrated that circadian clock regulators play important roles in maintaining muscle mass and proper function. We found that Rev-erba, negative regulator of the clock, inhibits muscle cell proliferation, differentiation and muscle regeneration. As Rev-erba is a "druggable" ligand-modulated nuclear receptor, we will test whether inhibiting this protein by its specific antagonist, SR8278, can augment muscle regenerative abilities to improve muscle dystrophy in an animal model of DMD. These studies will determine the potential therapeutic applications of a Rev-erba inhibitor as a new ameliorative strategy to treat patients with muscular dystrophies.

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

Summary The genotoxicity of TDP-43, an hnRNP family protein primarily involved in RNA processing (but also binds to DNA) whose aggregation/toxicity have been etiologically implicated in Amyotrophic Lateral Sclerosis (ALS) is not investigated although the diseases associated with TDP-43 pathology accumulate significant genome damage. Our preliminary data demonstrate that: (1) TDP-43 is required for efficient DNA double strand break repair (DSBR) in neuronal cells; (2) TDP-43 is recruited to DSB sites to stably interact with DSBR proteins, in neuronal cells; (3) TDP-43 depletion markedly increases DSB accumulation and sensitized human cells to DSB-inducing agents; (4) Nuclear-specific depletion of TDP-43 in neuronal cells cause increased DSB damage. (5) A strong correlation between TDP-43's

nuclear clearance and the accumulation of DSBs in ALS-affected human postmortem spinal cord tissue. Based on these, we hypothesize that loss of nuclear TDP-43 in ALS, causes deficient repair of DNA strand breaks in neurons promoting cell death and thus deficient repair of DSBs is a key etiologic factor in ALS and other TDP-43 pathologies. These will be comprehensively tested in this project by pursuing three Specific Aims using state of the art biochemical, cell culture including primary neuronal culture) and mouse and human tissue. Achieving our goals will lead to a major paradigm shift in our understanding of ALS pathology will open up new avenues therapeutic interventions.

Houston - Methodist Neurological Institute

Stanley Appel MD

RG	Immune Mechanisms in Amyotrophic Lateral Sclerosis.			
	$84,600.00	5/1/2015	4/30/2016	Year 2
	$84,600.00	5/1/2016	4/30/2017	Year 3

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

Houston - The University of Texas Health Science Center at Houston

Mariah Rose Baker Ph.D.

DG	Structural basis for EC coupling in the skeletal muscle CaV1.1 channel			
	$50,691.00	5/1/2015	4/30/2016	Year 2
	$50,691.00	5/1/2016	4/30/2017	Year 3

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

VIRGINIA

Richmond - Virginia Commonwealth University

Montserrat Samso Ph.D

RG Structural Analysis of RyR1 with Central Core Disease Mutations

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary This project employs cryo electron microscopy to get accurate 3D renderings of the ryanodine receptor (RyR), a calcium channel important for contraction of the voluntary muscles. These 3D renderings will enable understanding how this channel works normally and how abnormal function results in central core disease. This will be combined by measurement of the function of this channel in live cells. These methods will be then used to evaluate novel agents designed to correct RyR malfunction that could be used as a potential new therapy for central core disease.

WASHINGTON

Seattle - University of Washington

Jeffrey S Chamberlain Ph.D.

RRG Systemic Delivery of AAV vectors

$147,928.83	1/1/2015	12/31/2016	Year 1

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

Joel R. Chamberlain Ph.D.

RG16 DUX4 protein phenotypic effects in FSHD biopsies and in a mouse model of FSHD

$100,000.00	8/1/2016	7/31/2017	Year 1
$100,000.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary A roadblock in understanding the disease mechanism of FSHD has been the lack of a mouse model that recapitulates the muscle phenotypic changes associated with the disease. The sensitivity of muscle to the effects of DUX4 expression has hampered our ability to develop an animal model of FSHD that could give us key information about how the genetic changes lead to muscle weakness and potentially provide us points of intervention for therapy development. To address this problem we have developed a postnatal mouse model of FSHD based on muscle low-level expression of human DUX4 from the DUX4 promoter. DUX4 mice share muscle phenotypic features with human FSHD muscle that become more pronounced over time. This application will describe key advancements in our understanding of the effects of DUX4 protein expression, including the first localization of DUX4 protein in muscle and its physical association with muscle damage in both mouse and human tissue. We propose to use the mouse model and tools developed to characterize the origin of DUX4 protein expression and interrogate the molecular, biochemical, and functional changes in the DUX4 expressing mouse. In addition to linking expression of DUX4

protein to phenotypic changes in FSHD muscle, we will use what is learned from the mouse model characterization to guide our analyses of precious human biopsy samples and to inform testing of available and new potential therapies in animal models of disease.

Stephen D. Hauschka Ph.D.

RG New Regulatory Cassettes for Treating Diseased Muscle Tissues

$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.

Donghoon Lee Ph.D.

RG Magnetic Resonance Biomarkers for Effective Treatments for Muscular Dystrophy

$100,000.00	8/1/2015	7/31/2016	Year 1
$100,000.00	8/1/2016	7/31/2017	Year 2
$100,000.00	8/1/2017	7/31/2018	Year 3

Summary The goal of this project is to develop noninvasive magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) biomarkers that identify underlying tissue and cellular events associated with degeneration and regeneration processes in muscular dystrophy. Clinical assessments of muscular dystrophy routinely involve genetic, physiological, biochemical and histopathological methods, largely by surgical biopsy. Although they provide key information of muscle dysfunction, these methods are limited by their narrow sampling regions of interest and invasive nature of the procedure. We developed multi-parametric magnetic resonance (MR) approaches that extended evidence that MR can significantly facilitate noninvasive diagnosis and monitoring of muscle dysfunction. MRI based on changes in T2 weighted (T2w) imaging is commonly used to identify regions of pathology in muscle because of their sensitivity to a wide range of mechanisms. However, the T2w MRI alone is unable to identify specific cellular processes in the affected areas. Our new MR tools can quantitatively monitor muscle degeneration and regeneration processes in muscular dystrophy. In this project, a group of MR tools will be devised that identify specific markers for tissue and cellular changes with the disease progression and therapeutic treatments for muscular dystrophy. We will monitor the disease progression and identify biomarkers useful for effective monitoring of the therapy responses.