AUSTRALIA

Clayton - Monash University

Robert Bryson-Richardson Ph.D

RG16 — Evaluation of therapeutic approaches for FLNC myofibrillar myopathy

$90,800.00	2/1/2018	1/31/2019	Year 1
$89,101.00	2/1/2019	1/31/2020	Year 2
$94,061.00	2/1/2020	1/31/2021	Year 3

Summary We have generated zebrafish models for myofibrillar myopathy, due to mutation of Filamin C, that reproduce the key symptoms of protein aggregation and muscle fiber failure. We have identified that the formation of protein aggregates, due to the presence of the mutant protein, results in a reduction in functional protein as both mutant and functional protein become trapped in the aggregates. The breakdown of the muscle fiber results from the reduction in the amount of available functional protein. This suggests two therapeutic approaches, increasing the level of the functional protein and removing the protein aggregates. We will use drug treatments to promote the upregulation of protein degradation pathways in our disease models and determine their effectiveness at reducing the protein aggregates. We will also investigate drugs targeting pathways that control Filamin C protein levels. Our previous work has also demonstrated that another protein, BAG3, prevents the removal of the disease causing form of Filamin C from the body. We will therefore investigate drugs that inhibit BAG3 as a third possible therepeutic option. All three approaches will be tested in the zebrafish models to determine their effectiveness at reducing disease severity. The most promising compounds from each approach will be then evaluated in a long-term study using zebrafish that have been genetically modified to match change found in patients.

Peter David Currie PhD

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

$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	2/1/2017	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?			
	$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.

Nigel George Laing Ph.D.

RG16	Evaluating gene therapy for McArdle's disease using a mouse model			
	$99,206.00	2/1/2017	1/31/2018	Year 1
	$50,206.00	2/1/2018	1/31/2019	Year 2

Summary McArdle disease is caused by when an important muscle enzyme that is required to break down muscle energy stores is missing. The disease causes muscle pain when exercising, and in severe cases, muscle weakness/wasting occurs and can limit daily activity. There is currently no cure for McArdle disease. We will use a mouse model with McArdle disease to test two potential treatment methods. Both methods will use a modified version of virus to deliver crucial genetic material. For the first method will deliver a normal version of the missing enzyme. Although very logical, this approach is likely to cause an immune reaction as the body of a McArdle disease mouse (or a human patient) will have never seen this enzyme before. Thus, we will also in a parallel set of experiments promote increase levels of an alternative version of the enzyme. This alternative enzyme is found in adult brain tissue and in foetal muscle, and it has the same function of the missing enzyme in skeletal muscles. We propose that due to this alternative brain version being so similar, it could restore enzyme function in the muscle if it is increased to sufficient quantities. These potential therapeutic strategies will be clinically applicable to patients and may also be applied to other muscle diseases.

Sydney - The University of Sydney

Michael L.H. Huang Ph.D.

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

BRAZIL

São Paulo - Fundacao Faculdade de Medicina

Natassia Vieira Ph.D

DG   Jagged1 as a genetic modifier of Dystrophin Deficiency

$60,000.00	8/1/2017	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

Michael A. Rudnicki PhD

RG15   Molecular regulation of satellite cell function

$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.

Toronto - The Hospital for Sick Children

James Dowling M.D., Ph.D.

RG15   Drug Discovery for RYR1-related myopathies

$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.

Dwi Kemaladewi Ph.D.

DG16 Interrogation of CRISPR/Cas9-mediated exon inclusion in MDC1A

$60,000.00	2/1/2018	2/1/2019	Year 1
$60,000.00	2/1/2019	2/1/2020	Year 2
$60,000.00	2/1/2020	2/1/2021	Year 3

Summary The research proposed here will explore the therapeutic potential of correcting disease-causing mutation in MDC1A using novel genome editing strategy to restore the expression of Laminin-alpha 2 protein, which is important in the stability and organization of skeletal muscle and nerves.

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	8/1/2017	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

Gary Armstrong Ph.D.

DG Synaptic mechanisms of neuronal dysfunction in genetic models of ALS

$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.

Heather D. Durham Ph.D.

RG16 — The role of nBAF chromatin remodeling complexes in ALS

$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.

Eric Alan Shoubridge Ph.D.

RG16 — Molecular and cellular pathogenesis of CHCHD10 in ALS

$95,723.00	2/1/2017	1/31/2018	Year 1
$95,723.00	2/1/2018	1/31/2019	Year 2
$95,723.00	2/1/2019	1/31/2020	Year 3

Summary Mitochondrial dysfunction has been implicated in some familial forms of ALS, but the evidence for mitochondrial involvement has not been compelling. In the last two years mutations in CHCHD10, which codes for a bona fide mitochondrial protein, have been reported by several groups internationally in patients with ALS, FTD and other motor neuron diseases. The disease is dominantly inherited, as are most familial forms of ALS, but the function of the gene remains completely unknown. This project aims to identify the normal function of CHCHD10 and elucidate the mechanism of the mutant forms of the protein in motor neurons derived from patient cells and in a zebrafish model of the disease.

Montréal - Centre Hospitalier de l'Université de Montréal

Alex Parker Ph.D

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

$97,735.00	2/1/2017	1/31/2018	Year 2
$97,735.00	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	2/1/2017	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

| $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 | 2/1/2017 | 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 - The Cyprus Institute of Neurology & Genetics

Kleopas A. Kleopa M.D.

RG16 Expanding the gene therapy approach for treating CMT1X

| $57,794.00 | 2/1/2017 | 1/31/2018 | Year 1 |
| $62,205.00 | 2/1/2018 | 1/31/2019 | Year 2 |

Summary The goal of this project is to advance and expand our gene therapy approach already developed in a previously funded MDA project to treat the X-linked form of Charcot-Marie-Tooth Disease. Using mouse models of this disease we have demonstrated that by a lumbar injection of a viral vector carrying the normal gene that is mutated in patients we can achieve expression of the normal protein in multiple nerves leading to improvement of the peripheral nerve pathology and motor performance in this model. Here we would like to overcome further issues that are important to expand this approach. Since we only used a single injection of the vector so far we will examine whether repeated injections can lead to higher expression rates. In addition, we will test whether treatment at later stages of the neuropathy in the disease model could provide similar benefit as the early treatment we used so far, a very relevant question for treating patients at various stages of the disease. We furthermore need to validate our method in a large animal model to prove that sufficient distribution of the vector can be achieved in a scale relevant for patients. Finally, we will develop a gene repair method for a subset of patients who may harbor mutated proteins that could negatively interact with the normal protein delivered by the vector, as suggested by our recent results. Overall, this project will advance the translation potential of gene therapy for this and other forms of inherited neuropathy.

FRANCE

Nantes - University of Nantes, INSERM UMR 1089

Caroline LeGuiner MD, PhD

RG16 Gene therapy for the cardiac disease in Duchenne Muscular Dystrophy

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary The aim of our program is to deliver preclinical data that will enable translation to the treatment of the cardiac disease in Duchenne patients by gene therapy. We are two teams committed (George Dickson, PhD, University of London, UK and Philippe Moullier, MD, PhD/Caroline Le Guiner, PhD University of Nantes, France) to this 3-year program. What, we believe, makes our program competitive is: (i) our combined expertise in gene therapy for Duchenne disease; (ii) our direct access to critical animal models among which the unique DMDmdx rat exhibiting a similar cardiac phenotype as to the one described in Duchenne patients; (iii) the direct access to multiple core facilities specialized in translational gene therapy programs. We will address the therapeutic potential of 2 murine mini-dystrophin genes that are expected to behave differently in vivo. They will be compared along side a near normal size dystrophin resulting from correction by exon skipping. We will use the viral vector AAV9 (known for its heart tropism) in three animal models: (i) the mdx mouse to mainly investigate the molecular interactions of the mini-dystrophin candidates with their expected physiological partners; (ii) the DMDmdx rat to mainly assess the functional cardiac stabilization/recovery by EKG and 2D-echocardiography; and (iii) the nonhuman primate to determine the optimal AAV9 dose after selective intracoronary injections and molecular determination of gene transfer efficacy.

ITALY

Rome - Fondazione Telethon

Maria Pennuto Ph.D.

RG16 Targeting activity-regulated (dys)function of androgen receptor in SBMA neurons

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary A mutation in the androgen receptor (AR) causes selective lower motor neuron degeneration with an unknown mechanism in spinobulbar muscular atrophy (SBMA). We will test the hypothesis that the

function of AR is regulated by neuronal activity through direct modification of AR itself, and that this process is altered by the mutation that causes SBMA. This hypothesis is based on our preliminary data, showing that modification of mutant AR by a factor named cyclin-dependent kinase 2 (CDK2) enhances neurotoxicity. To test our hypothesis, we will pursue these goals: To identify AR as a factor whose function is regulated by neuronal activity. We will test the hypothesis that AR activation in neurons is regulated by their activity through direct modification of the disease protein, and that this level of regulation of protein function is altered by the mutation causing SBMA. To assess the role of CDK2-mediated polyQ-AR modification in SBMA. We will test the hypothesis that elimination of CDK2 reduces the toxicity of mutant AR by direct modification of the disease protein. To identify specific inhibitors for therapy development. We will assess the efficacy of specific inhibitors of CDK2 to identify compounds for therapy development.

Trieste - International Centre for Genetic Engineering and Biotechnology

Franco Pagani MD

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

$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.

NETHERLANDS

Nijmegen - Radboud University Nijmegen Medical Centre (2)

Erik Storkebaum Ph.D.

RG16   Unraveling mechanisms by which mutant tRNA synthetases cause CMT neuropathy

$99,000.00	2/1/2017	1/31/2018	Year 1
$94,500.00	2/1/2018	1/31/2019	Year 2
$97,500.00	2/1/2019	1/31/2020	Year 3

Summary   In Charcot-Marie-Tooth (CMT) disease, the nerve cables which connect the muscles and skin to the spinal cord degenerate. This leads to muscle wasting, impaired movement, and loss of sensation. Mutations in five distinct genes encoding tRNA synthetases all cause CMT. tRNA synthetases play an essential role in the production of proteins. Although some mutations may result in the loss of the normal function of these proteins, it has been shown that at least a number of mutations result in the acquisition of a toxic function that results in the impaired production of new proteins. In this proposal, we wish to determine whether or not all mutant tRNA synthetases cause disease through acquisition of a novel, toxic function. Furthermore, we want to test the hypothesis that all mutant tRNA synthetases cause impaired production of new proteins. These investigations form an essential first step towards the design of effective treatments for these diseases.

SPAIN

Barcelona - Universitat Pompeu Fabra

Pura Munoz-Canoves Ph.D.

RG16   Understanding and reversing muscle stem cell regenerative decline in DMD

$99,950.00	8/1/2017	7/31/2018	Year 2
$99,956.00	8/1/2018	7/31/2019	Year 3

Summary   The studies outlined in this proposal aim to answer fundamental questions on the biology of stem cells in skeletal muscle (satellite cells) in Duchenne Muscular Dystrophy (DMD). We want to understand why the capacity of satellite cells declines over the course of DMD progression, since this will help to envision new pathways to enhance or restore muscle regeneration in these patients, particularly at advanced age, when muscle architecture is disrupted, and loss of regenerative capacity is maximal. We would like to prove that the loss of regenerative capacity of satellite cells in dystrophic muscle is

an autophagy-dependent process and that promoting autophagy in these cells -via administration of natural compounds- can restore their regenerative properties even at advanced DMD stages. - Presently, there are no Agencies or Patients' Associations in Spain to submit nor fund this proposal. - Presently, there are no Agencies or Patients' Associations in Spain to submit nor fund this proposal.

Madrid - Centro de Investigaciones Biologicas-CSIC

Natalia Rodriguez Muela Ph.D.

DG15	Autophagy controls SMN protein degradation			
	$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.

UNITED KINGDOM

Edinburgh - University of Edinburgh

Lyndsay Murray Ph.D

RG16	Understanding and Exploiting the Therapeutic Time Window in Mouse Models of SMA			
	$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	8/1/2017	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 - Action Duchenne

Diana Ribeiro MSc, BSc

CG	Imaging in Neuromuscular Disease 2017 conference			
$2,200.00	11/1/2017	5/31/2018	Year 1

Summary   This international conference will be held in Berlin from Sunday 19th to Tuesday 21st of November 2017. The conference program will feature internationally-recognized keynote speakers highlighting developments and advances in all aspects of muscle imaging. At least 200 participants from the neuromuscular field are expected to attend. The Imaging in Neuromuscular Disease Conference will be organized by the MYO-MRI project, a consortium supported by COST, the European Cooperation in Science and Technology. MYO-MRI focuses on applications of MR imaging and spectroscopy techniques in neuromuscular disease and has enabled collaboration on MRI as an outcome measure and on pattern recognition for diagnostics and therapy development. The project has addressed the challenges of applying MR techniques in both clinical trials and everyday clinical settings. The Imaging in Neuromuscular Diseases Conference will highlight key findings from MYO-MRI and will discuss next steps of interest to academia and industries. The programme will feature plenary talks on the following topics; • Diagnostic Muscle Imaging • New Imaging Techniques • Quantitative Muscle Imaging with plenary sessions and invited abstracts on each.

London - Institute of Neurology, University College London

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

RG15	Understanding early-onset neuropathies using genome and transcriptome sequencing			
$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

Mary Margaret Reilly M.D.

HCTG	Muscle MRI in Charcot Marie Tooth disease .			
$329,573.75	12/1/2017	12/1/2018	Year 1
$355,109.46	12/2/2018	12/1/2019	Year 2

$316,631.54	12/2/2019	12/31/2020	Year 3

Summary The four most common types of Charcot Marie Tooth disease (CMT), which is the commonest inherited neuromuscular disease, are CMT1A (> 50% of all CMT), CMT1B, CMT2A and CMTX1 accounting for more than 90% of all CMT. A major barrier to clinical trials is that none of our current outcome measures can detect change adequately over 24 months. We have developed and published (in London, UK) a protocol to measure fat accumulation in thigh and calf muscles. Fat accumulation occurs in muscles which are denervated (i.e. muscles in which the nerves to the muscles are damaged as in CMT). In a study of 20 adults with CMT1A, we showed that calf muscle fat increased significantly over 12 months. This is the first responsive outcome measure to be identified in CMT1A over a 12 month period. We have now in a pilot study shown this protocol to be responsive in patients with CMT1A in another site (Iowa, US) and shown that the protocol can be reliably applied with central image analysis being done in London. We now aim to refine our protocol to include foot muscles as in very mild cases especially in younger people we noticed that calves may have no fat infiltration. We will study the refined protocol in children with CMT1A which is important as the disability in CMT1A starts in childhood and ideally treatment needs to begin in childhood. We will also extend our MRI protocol to the other 3 common types of CMT to validate the protocol in all the common types of CMT.

UNITED STATES

ALABAMA

Birmingham - The University of Alabama at Birmingham

Matthew Alexander Ph.D.

RG16 Therapeutic use of KPT-350 to block disease pathology in muscular dystrophy

$99,550.00	2/1/2018	1/31/2019	Year 1
$99,550.00	2/1/2019	1/31/2020	Year 2
$99,550.00	2/1/2020	1/31/2021	Year 3

Summary Duchenne muscular dystrophy (DMD) is an X-linked disorder that affects approximately 1:5000 live male births, and is the worldwide most common form of muscular dystrophy. DMD is caused by loss-of-function mutations in the DYSTROPHIN (DMD) gene from which patients are afflicted with muscle weakness, cardiac and respiratory problems. Current DMD treatments focus on prevention of muscle loss and respiratory assistance; however, there is no cure. In collaboration with Karyopharm Therapeutics, we have identified a compound (KPT-350) that blocks several of the symptoms associated with dystrophin-deficiency in the sapje zebrafish DMD model. Short-term and long-term dosing of sapje zebrafish with KPT-350 resulted in improved muscle function, reduced muscle damage, and overall extension of sapje lifespan. Recent published experiments in ALS mouse models have shown that KPT-350 improve muscle function and block fibrosis often associated with muscle wasting diseases. We have also generated strong pilot data showing that KPT-350 short-term (2 month) dosing improves muscle histology and pathology in dystrophic mdx (DBA2J) mice. The major objectives of this project are to define the optimal doses of KPT-350 and fully characterize the therapeutic potential of KPT-350 in pre-clinical dystrophic mouse studies. We will also evaluate the long-term consequences of KPT-350 dosing with an additional emphasis on molecular pathways affected by KPT-350 treatment.

Michael Miller Ph.D.

RG15 Postnatal Origin of Amyotrophic Lateral Sclerosis

$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

$94,107.00	8/1/2017	7/31/2018	Year 2
$92,504.00	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

Oro Valley - ICAGEN-T

Paul August Ph.D.

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

$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.

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

Rita Sattler Ph.D.

RG   Role of synaptic dysfunction in C9orf72-mediated pathogenesis

$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.

Scottsdale - Iron Horse Diagnostics, Inc.

Andreas Jeromin Ph.D.

MVPNEW | Validation of biomarkers to support ALS drug development

$16,500.00	12/4/2017	5/3/2018	Year 4

Summary Amyotrophic Lateral Sclerosis (ALS, or Lou Gehrig's disease) is a progressive and fatal neuromuscular disease marked by limb weakness, swallowing and breathing problems, and muscle atrophy. The average time to diagnose ALS is approximately 13 months. Iron Horse Diagnostic is preparing to launch the first biologic ALS diagnostic test. A simple and inexpensive test that will yield results within days, not months, allowing for quicker therapeutic intervention for the patient. During research for our diagnostic test, Iron Horse began developing indicators (biomarkers) of ALS disease progression. These prognostic tests can be used to support therapeutic clinical trials and speed the development of life saving drugs for ALS. Iron Horse is requesting funding to finalize development of our prognostic biomarkers and to launch the tests to the market. These tests will be essential tools to enhance drug development efforts in ALS.

Tucson - Arizona Board of Regents, University of Arizona

Daniela Zarnescu Ph.D.

RG16 | Metabolic dysregulation in ALS

$99,881.00	8/1/2017	7/31/2018	Year 2
$99,986.00	8/1/2018	7/31/2019	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)

Gino Cortopassi Ph.D.

RG16 | A drug for mitochondrial biogenesis in humans for muscle disease

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary A critical step for the approval of drug therapy for muscle disease is FDA approval. There are currently no FDA-approved drugs for mitochondrial muscle diseases (myopathies) in the USA. We show that the drug DMF increases the number and function of mitochondria in mice and humans. This drug DMF is the ONLY FDA-approved drug in the USA and Europe that has been shown to increase mitochondria, it is unique. We propose to test it in an animal model of mitochondrial muscle disease, and of Duchenne's muscular Dystrophy. If the drug is effective these are crucial data to enable the FDA allowing us to try DMF for the treatment of Mitochondrial Myopathy, or of Duchenne's Muscular Dystrophy. Because DMF is Already Approved for use in humans, this is a much shorter path to the clinic than starting with a completely new drug molecule, and gets drug to patients who need it faster.

Ricardo Anibal Maselli M.D.

RG | Replacement Therapy for Congenital Deficiency of Endplate Acetylcholinesterase

$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.

Duarte - Beckman Research Institute of the City of Hope

KE MA MD, PhD

RG15 The Therapeutic Potential of Circadian Clock Modulators in Muscular Dystrophies

$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.

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	8/1/2017	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.

Fatima Gasset-Rosa PhD

DG16 Intracellular TDP-43 liquid-liquid demixing, aggregation and spread in ALS

$60,000.00	2/1/2018	1/31/2019	Year 1
$60,000.00	2/1/2019	1/31/2020	Year 2
$60,000.00	2/1/2020	1/31/2021	Year 3

Summary Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron loss leading to fatal paralysis. Seminal findings with profound implications in neurodegeneration have recently highlighted 1) liquid-liquid de-mixing of proteins as a mechanism for intracellular compartmentalization 2) that repetitive phase separation can nucleate protein misfolding within those compartments, and 3) cell-to-cell spread within the mammalian nervous system of self-templating,

misfolded protein aggregates of a-synuclein, tau or SOD1. Recognizing TDP-43 aggregation to be nearly universally found in ALS and apparent spread of ALS from focal site, the combination of these discoveries supports a possible trans-cellular transmission of a TDP-43-dependent proteinopathy as a component of TDP-43 pathogenesis. I have identified two ways in which TDP-43 de-mixing and aggregation can be achieved at high efficiency within neuronal-like cells or in vitro. Here, I propose to determine the dynamics and composition of TDP-43 liquid-liquid de-mixing and conversion to solid aggregates. Also, assess if de-mixed/aggregated TDP-43 propagates from cell-to-cell. Finally, I will test if transmission of TDP-43 aggregation from cell to cell contributes to disease spread in mice. These approaches will elucidate mechanisms of TDP-43 de-mixing and aggregation, hallmark of ALS and may enable therapeutic strategy(ies) to prevent the formation of what may be disease initiating TDP-43 aggregation.

Jie Jiang Ph.D.

DG16	Role of C9orf72 loss of function in ALS caused by GGGGCC repeat expansions			
	$60,000.00	2/1/2017	1/31/2018	Year 1
	$60,000.00	2/1/2018	1/31/2019	Year 2
	$60,000.00	2/1/2019	1/31/2020	Year 3

Summary   Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative disorder, characterized by the selective loss of motor neurons leading to paralysis. Expanded GGGGCC hexanucleotide repeats in a non-coding region of the C9orf72 gene are the most common genetic cause of ALS and frontotemporal dementia (FTD), another neurological disease characterized by behavioral and language changes. Proposed pathogenic mechanisms include reduction in endogenous C9orf72 protein function and/or a toxic gain of function from the repeat-containing RNAs mediated either by sequestration of RNA binding proteins or by translation of those RNAs into aberrant dipeptide repeat proteins. My previous efforts have established that C9orf72 repeat expansions contribute to ALS/FTD disease pathogenesis in part by a gain of toxicity mechanism and that reduced expression of C9orf72 in mice alone is insufficient to produce ALS/FTD. Additional efforts have suggested that loss of C9orf72 alters function of microglia, the immune cells in the central nervous system, and that this may be a contributor to neurodegeneration in C9orf72 expansion carriers. This proposal will use genetics in mice to determine whether C9orf72 loss of function synergizes with gain of toxicity to produce, to understand the role C9orf72 in microglia, and establish its contributions to ALS/FTD. The outcome of this proposal will guide direction of therapeutic development for ALS/FTD caused by C9orf72 repeat expansions.

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	8/1/2017	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	2/1/2017	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 Regents of the University of California, San Diego

Jordan Blondelle PhD

DG16   Role of Cullin-3 targeted protein turnover in skeletal muscles

$60,000.00	8/1/2017	7/31/2018	Year 1
$60,000.00	8/1/2018	7/31/2019	Year 2
$60,000.00	8/1/2019	7/31/2020	Year 3

Summary   Protein degradation is an essential mechanism by which a plethora of cellular processes is regulated. The Cullin family of ubiquitin E3-ligases is a key component of that system. Once incorporated into complexes, Cullins are able to bind and mark unwanted proteins for degradation. Recently, mutations in several binding partners of Cullin-3, a Cullin protein family member, were found in patients with severe forms of nemaline myopathy. These findings suggest a specific and important function for Cullin-3 targeted protein degradation during muscle development and in muscle physiology. While intensively investigated in the context of cancer, very few data are available regarding the role of Cullin-3 in muscles. Using mutant mice depleted of Cullin-3 in muscles, we unraveled its absolute necessity for postnatal survival. In this project, we will ask how the loss of Cullin-3 is affecting muscle development, structure and function. We expect to find new target proteins of the Cullin-3 complex that would be misregulated in absence of Cullin-3 and responsible for the deleterious phenotype observed in mice. Indeed, the overall aims of our project are to decipher, for the first time, how protein degradation mediated by Cullin-3 is relevant for muscle physiology and to give us insights regarding the pathological mechanisms leading to nemaline myopathy in patients.

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	2/1/2017	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	2/1/2017	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	8/1/2017	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	2/1/2017	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	8/1/2017	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

$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

Justin Ichida Ph.D.

RG   The Role of C9ORF72 Protein Function in Amyotrophic Lateral Sclerosis

$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	2/1/2017	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

Thomas Rando M.D., Ph.D.

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

$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 | 8/1/2017 | 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.

Antoine de Morrée Ph.D.

DG　　　　　Towards FSHD Therapeutics: Understanding Polyadenylation Site Choice

| $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.

Maya Maor

DG16　　　　　SSSI Fellowship - Uncovering epigenetic changes in response to microtubules dysfunctions in ALS

| $20,000.00 | 10/1/2017 | 9/30/2018 | Year 1 |
| $20,000.00 | 10/1/2018 | 9/30/2019 | Year 2 |

Summary

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	5/1/2017	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	5/1/2017	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.

Marta Margeta M.D., Ph.D.

RG16   Dysregulation of redox balance in autophagic vacuolar myopathies

$100,000.00	2/1/2018	1/31/2019	Year 1
$100,000.00	2/1/2019	1/31/2020	Year 2

Summary   Autophagy is a fundamental metabolic process with many important cellular functions, one of which is to degrade damaged cellular organelles and misfolded protein aggregates that can lead to cell dysfunction. Evidence of autophagy dysregulation is present in a number of inherited and acquired skeletal myopathies, including Pompe disease / acid maltase deficiency (a lysosomal storage disorder), sporadic inclusion body myositis, centronuclear myopathies, and inherited as well as drug-induced autophagic vacuolar myopathies; however, the underlying molecular and cellular mechanisms are poorly understood. The long term goal of the proposed research is to elucidate how autophagy dysregulation impairs skeletal muscle function and leads to muscle injury. The specific objective of the current application is to use in vitro and in vivo models of drug-induced autophagic myopathy to establish how dysregulation of the cellular redox balance that follows autophagy inhibition affects skeletal muscle biology. By elucidating downstream effects of autophagy impairment, the proposed research will facilitate development of pharmacologic therapies for inclusion body myositis and other autophagic myopathies, which currently lack successful treatment.

COLORADO

Aurora - University of Colorado Denver, AMC and DC

Alexander Polster Ph.D.

DG16   Structure and Function of Excitation-Contraction Coupling Proteins

$60,000.00	2/1/2017	1/31/2018	Year 1
$60,000.00	2/1/2018	1/31/2019	Year 2
$60,000.00	2/1/2019	1/31/2020	Year 3

Summary   Electrical signals produced by the nervous system trigger muscle contraction. This process, termed excitation-contraction (EC) coupling, depends on two key proteins: the dihydropyridine receptor

(DHPR) which is located in the membrane surrounding the muscle cell, and the type 1 ryanodine receptor (RyR1) located inside the cell. Mutations in these proteins result in serious muscle diseases in humans, including hypokalemic periodic paralysis (HypoPP) and central core disease (CCD). A recently identified adaptor protein (Stac3) plays an important role during muscle development and contributes essentially in signal transmission during EC coupling by binding to the DHPR. In this proposal, by employing a combination of molecular biological, optical and electrophysiological methods we will investigate how the DHPR, RyR1 and Stac3 interact with one another and why mutations cause these human muscle diseases. Information obtained in the course of this study may provide valuable information applicable to the study and treatment of degenerative and episodic muscle diseases.

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	2/1/2017	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	2/1/2017	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)

Marshall Hogarth Ph.D.

DG16   Targeting the lipid accumulation pathology in LGMD2B

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

$60,000.00	2/1/2018	1/31/2019	Year 2
$60,000.00	2/1/2019	1/31/2020	Year 3

Summary   Limb girdle muscular dystrophy type 2B (LGMD2B) is a progressive muscle wasting disease caused by mutations in the gene coding for dysferlin. Gradual replacement of muscle with fat is a feature of the pathology in both LGMD2B patients and dysferlin-deficient mice. Loss of muscle function in LGMD2B patients and mice is correlated with the fatty conversion of muscle, which suggests that the processes leading to fatty muscle conversion are significant to disease pathogenesis and are thus potential therapeutic targets. However, little is known about the mechanism of the fatty conversion and how the absence of dysferlin disposes muscle into this pathway. Eccentric exercise is known to hasten the disease progression in LGMD2B patients. We have mimicked this process using a muscle injury model which causes fat accumulation selectively in dysferlin-deficient muscle, but not in healthy controls. In this model the extent of fat accumulation is directly proportional to the degree of muscle injury we induce. In parallel, we have also developed a mouse model where age-dependent fat conversion of dysferlin deficient muscle is significantly reduced. This study aims to use the above approach and mouse model we have developed to understand the mechanisms underlying the fat replacement of LGMD2B muscle and identify means to intervene with this mechanism for therapeutic benefit.

James Novak Ph.D.

DG16   Factors that limit exon skipping efficacy in Duchenne muscular dystrophy

$60,000.00	2/1/2017	1/31/2018	Year 1
$60,000.00	2/1/2018	1/31/2019	Year 2
$60,000.00	2/1/2019	1/31/2020	Year 3

Summary   The major challenges facing exon-skipping therapies for Duchenne muscular dystrophy (DMD) are to improve efficiency of delivery of the antisense agents to skeletal/cardiac musculature and to determine the extent of functional improvement achieved by restoration of a given amount of dystrophin. Our current work shows that that penetration of morpholino antisense agents is linked to muscle regeneration, a prominent feature of DMD pathology. We show that morpholino primarily localizes within muscle fibers during the brief period of repair when differentiated myogenic cells fuse into them. This prompts us to test whether exercise-induced muscle regeneration improves morpholino uptake and exon skipping. Up to now, work has been limited to the original dystrophic mouse model, whose validity as a preclinical surrogate of DMD is widely debated. We propose to determine how the different disease pathology seen in the DBA/2J-mdx mouse may influence drug uptake, localization and efficacy by comparison with the original Bl10-mdx model. In addition to the morpholino, we propose to investigate the entry of tricyclo-DNA antisense, another promising exon skipping agent, which we suspect enters by additional or alternative mechanisms. Our comparative experimental design will clarify the various features of disease pathology that contribute to efficient delivery and dystrophin restoration of leading antisense agents in order to improve this valuable therapeutic strategy for DMD.

Washington - The George Washington University

Henry Kaminski MD

RG16   Targeted Complement Inhibition for Myasthenia Gravis

$145,841.00	2/1/2017	1/31/2018	Year 1
$150,206.00	2/1/2018	1/31/2019	Year 2
$71,140.00	2/1/2019	1/31/2020	Year 3

Summary   Myasthenia gravis causes severe weakness which may be life-threatening. In the majority of patients antibodies against the acetylcholine receptor and limit nerve-muscle communication by activation of a system called complement. We have a novel technology that inhibits complement only at the nerve-muscle communication point and preserves the role of complement in fighting infections.

Linda L Kusner Ph.D

RG16   INHIBITORS OF APOPTOSIS IN MYASTHENIA GRAVIS.

$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.

FLORIDA

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

Michael Benatar Ph.D., M.D.

CRNG15   Clinical Research in ALS and Related Disorders for Therapy Development (CReATe)

$150,000.00	3/1/2017	2/28/2018	Year 1
$150,000.00	3/1/2018	2/28/2019	Year 2

Summary   During the course of ALS, patients require input and assistance from multiple health professionals, and as a result, multi-disciplinary clinics are, perhaps, the key resource for ongoing management and care. These clinic visits take several hours and substantial clinical data is routinely collected (e.g. neurological examination, ALSFRS-R, quantification of respiratory muscle strength, etc.). Much of this data is identical to the assessments performed at specialized "research visits," which utilize distinct health record systems and burden patients with profound weakness to attend both clinic and research appointments.

Stephan Zuchner M.D.

RIG   A PATIENT-CENTRIC DATA RESOURCE FOR CMT GENETICS (DiRECT)

$219,967.00	12/1/2017	1/31/2018	Year 1
$110,000.00	2/1/2018	1/31/2019	Year 2
$55,000.00	2/1/2019	1/31/2020	Year 3

Summary   Increasingly, many of our efforts to help patients are driven as much by data science, IT, and computing abilities of genomic data as they are by wet lab procedures. For inherited diseases like Charcot-Marie-Tooth disease (CMT) the ability to analyze, archive, compare, easily share genomic data is a growing part of the necessary work. Depending on this ability are: 1) our diagnostic capacities; 2) guidance for functional studies that lead to drug discoveries; and 3) increasingly the enrollment and patient selection process necessary for well-designed trials. An open CMT genetics data resource is very important to expedite the process from gene identification to therapeutic impact. Together with the Inherited Neuropathy Consortium, we have successfully developed genomic data infrastructure (such as the GEM.app/ GENESIS computing platform) that has been widely used by CMT researchers over the past five years. With funding from MDA such resources will be expanded significantly and made available to even more CMT researchers in the US and around the world to share data in a secure way and to build the foundation for future therapeutic trials.

Gainesville - University of Florida

Andrew Berglund Ph.D.

RG16   Impeding transcription of the toxic RNAs of myotonic dystrophy

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   The goal of this research is to develop small molecules that reduce or eliminate the toxic RNA that causes myotonic dystrophy. If successful, this approach can potentially be used in combination with other therapeutic strategies to relieve the suffering of myotonic dystrophy patients.

Vijayendran Chandran Ph.D

RG16   Integrating omics approaches to identify biomarkers in Friedreich's Ataxia.

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   Currently, there is no effective approved treatment for Friedreich's ataxia (FRDA). Molecular biomarkers are important to assess clinical trial out comes, to measure the progression of the disease and to evaluate the most effective therapeutic agents. Currently, there are no high confidence molecular biomarkers associated with FRDA for clinical and preclinical assessment. The absences of good molecular biomarkers in FRDA can be attributed to the non-availability of affected tissues during the earlier and late period of the disease progression. However, utilizing appropriate animal models will provide us the opportunity to identified robust and reproducible biomarkers directly from the tissue that is affected. We have developed an inducible and reversible mouse model for FRDA, which allows us to control the onset and progression of the disease. We were able to reverse the acceleration of disease progression even after significant motor dysfunction was observed. Since, our novel FRDA mouse model exhibits various symptoms parallel to FRDA patients, we plan to screen and identify high confidence prognostic and predictive molecular biomarkers associated with FRDA disease progression.

Manuela Corti Ph.D.

RG16   AAV-mediated Gene Therapy in Friedreich's Ataxia

$99,617.00	2/1/2017	1/31/2018	Year 1
$99,817.00	2/1/2018	1/31/2019	Year 2
$99,520.00	2/1/2019	1/31/2020	Year 3

Summary   The objective of this proposal is to develop a treatment strategy for Friedreich's Ataxia (FA), one of the most common forms of ataxia. Specifically, our research plan focuses on the correction of both the cardiac and neurological degeneration found in the disease. These changes are due to harmful changes in the frataxin gene. Currently, the only treatments available are designed to lessen symptoms and do not address the fundamental cause of FA, which is reduced levels of frataxin. Therefore, the primary objective of this program is to establish a high-impact treatment for FA by correcting the level of frataxin the cell using a vehicle to carry a fully functioning frataxin gene in to cells in the heart and nervous system. This proposal will specifically answer important mechanistic questions in a new FA mouse model, which has many of the symptoms of the human patients. First, we will identify the best route of delivery for the frataxin gene in the nervous system by comparing three different strategies for injecting the vector. Second, we will test the safety of repeated delivery of the frataxin gene vector in combination with medications that will prevent reactions against the frataxin protein and the vector components. Completion of this project will be an important milestone in the development of a treatment strategy that will dramatically improve quality of life for FA patients.

Darin Falk PhD

RG16   Identifying mechanisms of respiratory failure in Pompe disease

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   Pompe disease is a neuromuscular disorder characterized by the inability to break down glycogen in tissue through lack of the enzyme acid alpha-glucosidase (GAA). Subsequent glycogen accumulation ultimately causes muscle function and cardiorespiratory failure before 12 months of age in early-onset patients. A current challenge in developing therapies to treat Pompe patients is lack of a suitable model that accurately reflects the severity of heart and muscle impairment that is displayed in human patients. To enhance our ability to develop impactful therapies, we have recently developed a new rat model of Pompe disease. This model displays the hallmark disease traits with greater resemblance to the human Pompe population. Our initial characterization has determined that severe cardiac and respiratory impairment leads to early mortality in this novel model, and closely mimics the disease in humans. Our goal is to use this advanced model to characterize the mechanisms that lead to cardiorespiratory decline and evaluate the ability of current and emerging therapies to preserve cardiac and respiratory function and improve survival outcomes.

David W Hammers Ph.D.

DG16   Targeting senescent stroma in DMD

$60,000.00	2/1/2018	1/31/2019	Year 1
$60,000.00	2/1/2019	1/31/2020	Year 2
$60,000.00	2/1/2020	1/31/2021	Year 3

Summary   Duchenne muscular dystrophy (DMD) is a devastating and lethal genetic disease that results in the severe degeneration of functional skeletal muscle and replacement with non-functional connective tissue. It is this replacement of muscle tissue that results in the loss of ambulation and ventilation amongst DMD patients, therefore understanding the mechanisms responsible for and the development of therapeutic to prevent this fibrotic replacement are a major need for this patient population. The current proposal investigates the hypothesis that senescence, a stable cellular state that results in persistence and secretion of repair-inhibiting factors, of muscle interstitial cells are involved in the fibrotic replacement of muscle associated with muscular dystrophies. To accomplish this, senescent cell populations of dystrophic mouse muscle will be investigated, where population size, growth, and expression profiles will be evaluated. Additionally, the involvement of the pro-oxidant enzyme, NOX4, in the development of senescence will be assessed by both genetic knockout and preclinical testing of a NOX1/4 inhibitor in dystrophic mice. The results from these studies will provide detailed information about a novel mechanism of muscle fibrosis and further the investigation of anti-fibrotic therapies for the treatment of DMD.

Laura P.W. Ranum Ph.D.,

CG   9th International Conference on Unstable Microsatellites and Human Disesase

$7,500.00	3/1/2018	3/30/2018	Year 1

Summary   The 9th International Conference on Unstable Microsatellites and Human Disease (UMHD9) will be held at the Hotel La Palma in Capri Italy from January April 21-26th, 2018. This meeting is unique in that it brings together investigators studying a variety of repeat disorders, addressing different aspects, using different approaches and model systems. We have developed an innovative and cutting edge scientific program that will emphasize emerging themes in pathogenic and mutagenic mechanisms of the repeat disorders and harness this knowledge for therapeutic benefit. We have worked hard to ensure a wide diversity of participants, the inclusion of scientists from different institutions, geographical areas and researchers at different stages of their careers. This fosters tremendously productive discussions on the common features and differences in these repeat disorders. For the first time this year we will be holding a special breakfast session for Women and Minorities in Science. This "Meet the Leaders Breakfast" will provide a networking opportunity and discussion of topics related to women and minority scientists in the field of repeat disorders. Research opportunities will be discussed along with the challenges that could be faced, and strategies to overcome the barriers they may encounter, with more senior scientists who have faced similar challenges.

Laura P.W. Ranum Ph.D.,

RG16   Molecular characterization & antibody therapy in a novel C9orf72 BAC mouse model

$73,356.00	8/1/2017	7/31/2018	Year 2

Summary   There are currently no effective treatment strategies for the large group of neurodegenerative diseases caused by repeat expansions within the human genome. We have discovered a new mechanism by which these disease-causing repeats produce an unexpected and previously unknown category of proteins without using the normal protein production start signal. We have shown the presence of these proteins within human autopsy tissue from patients with the most common cause of familial amyotrophic lateral sclerosis/frontotemporal dementia (C9orf72 ALS/FTD). Additionally we and others have shown that the expanded repeats are made into RNA in both sense and antisense directions in these patients. The goals of this project are to understand when and where these RNA and proteins are produced in ALS/FTD and how they contribute to disease. To accomplish these goals we will use a new mouse model we developed which recapitulates the molecular and disease features of C9-ALS/FTD. We will also test new treatment strategies that target the proteins in the brains of these mice to see if treatments increases survival and decrease disease symptoms. These studies will result in a greater understanding of the mechanisms behind ALS/FTD and may provide new treatments strategies urgently needed for ALS/FTD patients.

Maurice Swanson Ph.D.

RG16	Novel Mouse Knockin Models for Myotonic Dystrophy			
	$100,000.00	2/1/2017	1/31/2018	Year 1
	$100,000.00	2/1/2018	1/31/2019	Year 2
	$100,000.00	12/1/2019	1/31/2020	Year 3

Summary   The neuromuscular disorder myotonic dystrophy (DM) is the most common form of adult-onset muscular dystrophy. DM is an inherited disease caused by the expansion of DNA simple sequence repeats, or microsatellites, in two different genes, DMPK and CNBP. When transcribed, the resulting repeat expansion RNAs alter the normal functions of the MBNL and CELF RNA binding proteins, which play a central role in developmental gene expression, resulting in the appearance of fetal gene products in adult tissues and disease. A primary goal of the proposed research is to generate and characterize new mouse models for both types of DM (DM1, DM2) to investigate the molecular basis for the clinical differences and similarities observed between these disease forms and to reproduce the multi-system phenotypes of DM1 and DM2. The second objective is to use these DM1 and DM2 mouse models to test a new therapeutic strategy that involves blocking the production of mutant DMPK and CNBP expansion RNAs at the transcriptional level using small molecule drugs, which cross the blood-brain barrier, to reverse the effects of this disease in all tissues, including the central nervous system.

Lukasz Jakub Sznajder Ph.D.

DG16	Pre-mRNA Misprocessing in Myotonic Dystrophy and Amyotrophic Lateral Sclerosis			
	$60,000.00	2/1/2018	1/31/2019	Year 1
	$60,000.00	2/1/2019	1/31/2020	Year 2
	$60,000.00	2/1/2020	1/31/2021	Year 3

Summary   Myotonic dystrophy type 2 (DM2) and C9orf72-linked amyotrophic lateral sclerosis/frontotemporal dementia (C9-ALS/FTD) are distinct hereditary diseases caused by the expansion of simple sequence repeats in two different human genes. DM2 is a highly variable disease that is characterized by muscle excitability, proximal limb muscle weakness, tremors and varying degrees of progressive cognitive impairment. In contrast to DM2, ALS is an ultimately fatal degenerative disorder of motor neurons that results in paralysis and muscle atrophy. Currently, no effective therapy for either disease exists. Interestingly DM2, C9-ALS/FTD and five other neurological diseases share some inherited features causing selective RNA mis-processing. The objective of the proposed research is to apply next-generation sequencing and other technologies to develop novel and disease-specific blood biomarkers for known expansion disease genes and generate an accessible microsatellite expansion gene discovery tool for idiopathic diseases, including the more common sporadic form of ALS.

Rebecca Willcocks Ph.D.

DG	MRI, MRS, and functional characterization of the arm in DMD			
	$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	8/1/2017	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	8/1/2017	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	2/1/2017	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

Anita Hargrave Corbett Ph.D.

RG16   Understanding Pathology in Oculopharyngeal Muscular Dystrophy (OPMD)

$99,998.00	8/1/2017	7/31/2018	Year 2
$100,000.00	8/1/2018	7/31/2019	Year 3

Summary   The goal of this proposal is to understand why there is pathology in specific muscles in the disease, oculopharyngeal muscular dystrophy (OPMD). We have created new mouse models of OPMD to study the disease and optimize theraputic approaches. In the proposed work, we utilize these mouse models to ask specific questions about why the disease impacts muscle and understand how to best treat patients.

Jonathan D. Glass MD

RRG2   2016 Night of Hope event - Clinical Research in ALS (CRiALS)

$137,696.00	1/1/2018	12/31/2018	Year 1

Summary   The goal of this work is to create a comprehensive database containing demographic, clinical, and laboratory information on ALS patients seen and cared for at the Emory ALS Center. These clinical data will be connected to biospecimens from these same patients, including blood, DNA, spinal fluid, and skin cultures for research into the causes and drivers of ALS. In addition, biosamples will be collected from a control population that will include spouses, relatives and unrelated people. All of these data will be stored in a comprehensive database for data analysis and specimen retrieval. These specimens and connected clinical data will be used for local research projects as well as shared with ALS investigators around the world as a valuable resource for scientific discovery.

ILLINOIS

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

Auinash Kalsotra Ph.D.

RG16   Elucidating the molecular basis for cardiac dysfunctions in Myotonic Dystrophy

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   Myotonic dystrophy type I (DM1) is caused by an unusual mutation in which a small DNA segment of the mutated gene is repeated hundreds of times. The mutated gene, when copied into RNA, becomes toxic and particularly harmful because instead of its normal exit to the cytoplasm, the RNA with repeats gets trapped within the nucleus, which alters the normal function of many genes, not just the gene with the mutation. While the mutation affects multiple tissues, cardiac defects are the second leading cause of death amongst DM1 individuals; however, the molecular mechanism(s) responsible for the cardiac pathogenesis remain poorly understood. We have found that in DM1 diseased heart, the fetal non-muscle isoform of a previously unrecognized RNA binding protein is significantly up regulated. By forcing the expression of the non-muscle isoform of RBFOX2 in wildtype adult mouse heart, we have reproduced most of the cardiac dysfunctions observed in DM1, including arrhythmias and cardiac conduction defects. In this proposal, we will determine how the balance between muscle and non-muscle isoforms for RBFOX2 is achieved during normal heart development. We will further investigate how this regulation is disrupted in DM1 and why the selective expression of non-muscle RBFOX2 isoform triggers a cardiac disease phenotype. We will use newly generated mouse models, in vitro cell culture systems, and genome-wide approaches to answer these exciting questions.

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	8/1/2017	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 - Northwestern University - Chicago Campus

Evangelos Kiskinis PhD.

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

| $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.

Mattia Quattrocelli Ph.D.

DG16   Glucocorticoids in fiber repair and regeneration of dystrophic muscles.

$60,000.00	2/1/2017	1/31/2018	Year 1
$60,000.00	2/1/2018	1/31/2019	Year 2
$60,000.00	2/1/2019	1/31/2020	Year 3

Summary   Muscular dystrophies are characterized by chronic disruption of muscles, with consequent wasting of muscle. Normally, in response to muscle injury that disrupts the membrane around muscle, there is a repair complex that seals the damage. In dystrophic muscle, however, this process is impaired and in addition, dystrophic muscle do not regenerate as well as normal muscles. At present, glucorticoid steroids are the only pharmacological treatment for Duchenne Muscular Dystrophy. However, the side effects are prominent and the role of glucorticoids on the actual fiber repair process is still unknown. Moreover, the effects of glucorticoids on the stem cells in muscle are not well studied. With this project, we aim to define the effects of glucorticoids on muscle membrane repair and regeneration in dystrophic muscles using newly developed methods. We will first test the effects of pulsed and chronic administration of glucorticoids on fiber repair and resident stem cells of dystrophic muscles. We will then examine how glucorticoids counteract the negative effects of the TGFß molecular pathway on muscle repair and regeneration. Finally, we will study how glucorticoids and the novel genetic modifier Jagged converge towards beneficial effects on dystrophic muscles.

INDIANA

West Lafayette - Purdue University

Feng Yue Ph.D.

DG16   Therapeutic Potential of Pten Inhibition in Duchenne Muscular Dystrophy

$58,074.00	8/1/2017	7/31/2018	Year 1
$58,285.00	8/1/2018	7/31/2019	Year 2
$59,050.00	8/1/2019	7/31/2020	Year 3

Summary   Duchenne muscular dystrophy (DMD) is a genetic disorder caused by an absence of dystrophin, a protein that helps keep muscle cells intact. In DMD, the muscle is more susceptible to injury but

cannot keep up with repair, which eventually leads to muscle loss and weakness. The study in present proposal aims to develop potential therapies that promote regrowth of dystrophic muscle and, in turn, increase muscle strength. The strategy we developed involves inhibiting the action of a natural protein called phosphatase and tensin homolog (PTEN) that limits muscle cell growth. In healthy muscle, the level of PTEN is very low, but becomes very high in DMD muscles. Notably, lowering PTEN in healthy muscle is sufficient to allow muscle grow larger and stronger. Using a preclinical mouse model of DMD, we will first study whether inhibiting PTEN by genetic deficiency of PTEN gene in muscle cells could boost muscle growth, increase muscle strength in DMD mice. Moreover, we will develop a safe, high-efficiency pharmacological approach to specifically deliver a well-known Pten inhibitor to the skeletal muscle of DMD mice, and examine its effect on muscle functional recovery. These studies may lead to the development of novel therapeutic strategies for clinical treatment of DMD.

IOWA

Iowa City - The University of Iowa

Michael Shy M.D.

RIG   Inherited Neuropathy Consortium

$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.

Lori L. Wallrath Ph.D.

RG16   Suppression of lamin-induced muscle defects

$94,226.00	2/1/2017	1/31/2018	Year 1
$95,172.00	2/1/2018	1/31/2019	Year 2
$95,856.00	2/1/2019	1/31/2020	Year 3

Summary   Mutations in the human LMNA gene cause several types of muscular dystrophy, including Emery-Dreifuss muscular dystrophy. These diseases are characterized by restricted movement of the joints (elbows, ankles and neck), muscle weakness and wasting (especially of the upper arms and lower legs), and heart problems. There are currently no treatments for these types of muscular dystrophy. Our goal is to identify potential treatments. We are using the powerful genetics of a model organism (fruit fly) to identify potential drug targets. Our preliminary data show that we can restore muscle function in a fruit fly model of Emery-Dreifuss muscular dystrophy, demonstrating feasibility of this approach. We will test potential therapeutic drugs on the fruit fly model and on patient-derived cell cultures. Collectively, our studies will identify potential drug targets and test drugs that are predicted to restore muscle function based on our findings.

KANSAS

Kansas City - University of Kansas Medical Center Research Institute, Inc.

Jeffrey Statland M.D.

CRNG15   Facioscapulohumeral Muscular Dystrophy Clinical Trial Research Network

$423,926.00	4/1/2018	3/31/2019	Year 1
$423,926.00	4/1/2019	3/31/2020	Year 2
$423,926.00	4/1/2020	3/31/2021	Year 3

Summary   The overall goal of this proposal is to expedite the development of new therapies for facioscapulohumeral Muscular dystrophy (FSHD) by developing and maintaining a core FSHD Clinical Trial Research Network (CTRN). The establishment of a unifying model for the cause of FSHD means that it is possible to develop targeted treatments for FSHD. Laboratories are developing disease-directed therapies, and several pharmaceutical companies are actively investigating therapeutic

approaches to treat FSHD. Hand in hand with the work being done to develop new therapies comes the need for us as a research community to prepare for clinical trials. Establishing and maintaining an FSHD CTRN will allow us to: create an infrastructure of trained clinical evaluators using standardized approaches to measuring progression in FSHD; develop regulatory strategies which are stream-lined to accelerate therapeutic development; ensure all major stakeholders have a say in the drug development process, including researchers, patients, patient advocates, and industry; create a nationwide recruitment strategy to ensure all people with FSHD are represented; train the next generation of FSHD researcher; and run studies to build the tools for running high quality clinical trials, or testing new therapies. A functioning FSHD CTRN will be a resource for the whole FSHD research community.

KENTUCKY

Lexington - University of Kentucky Research Foundation

Haining Zhu Ph.D.

RG   FUS phosphorylation and its significance in ALS

$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	8/1/2017	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

Mohamed H. Farah PhD

RG16   Mechanisms of neuromuscular synapse plasticity induced by BACE1 inhibition

$100,000.00	2/1/2017	1/30/2018	Year 1
$100,000.00	2/1/2018	1/30/2019	Year 2
$100,000.00	2/1/2019	1/30/2020	Year 3

Summary Strategies aimed at promoting motor axon sprouting and functional restoration by reinnervation might be useful to treat motor neuron diseases. We previously discovered that knockout of beta secretase (BACE1), an enzyme that proteolytically processes amyloid precursor protein (APP), enhances peripheral nerve regeneration following sciatic nerve crush injury in mice. This prompted us to explore the possibility that pharmacological inhibition of BACE1 might be an effective means to encourage collateral sprouting of intact motor axons at an early stage of motor neuron disease. This possibility is attractive, given that the pharmaceutical industry is actively developing BACE1 inhibitors as candidate therapies for Alzheimer's disease, and is therefore amassing safety, efficacy, and biodistribution data on these molecules. Here, we present preliminary data to suggest that a small-molecule BACE1 inhibitor improves motor axon sprouting and restores nerve function in SOD1 mice, an aggressive model of motor neurons disease.

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

RG   C9orf72 ALS is caused by nuclear-cytoplasmic trafficking dysfunction

$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.

Baltimore - Johns Hopkins University-School of Medicine

Thomas Philips Ph.D

DG15   The role of monocarboxylate transporters in motor neuron disease

$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.

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

RRG2   Packard Center for ALS Research (Wings 2016) (Rothstein, JD)

$75,600.00   3/1/2018   2/28/2019   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 a affiliated with a Hopkins-based researcher who will participate in the Packard Center's collaborative process and whose proposal has been reviewed and approved by the Center's scientific advisory board. Any additional funding required for this project beyond that awarded by MDA's designated grant will be covered by the Robert Packard Center. Money received from this MDA designated grant will not be used to support Dr. Rothstein or his lab.

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

RIG   Answer ALS: A tool for data integration and characterization of disease networks

$186,494.00   5/1/2018   4/30/2019   Year 1

$181,753.00	5/1/2019	4/30/2020	Year 2
$181,753.00	5/1/2020	4/30/2021	Year 3

Summary   Amyotrophic Lateral Sclerosis (ALS) is a progressive disease of the nerve cells that leads to loss of voluntary muscle control, paralysis, difficulty speaking, swallowing and ultimately, breathing. Today, we recognize that ALS is unlikely to be one disease, rather, it may be a collection of subtypes and variants each of which requires a different approach for optimal treatment. Answer ALS is a nationwide consortium, which is assembling the largest and most comprehensive ALS dataset to date, in which clinical, behavioral and extensive cellular datasets will be collected from 1000 patients. This comprehensive dataset will allow, for the first time, in depth interrogation of the underlying disease mechanisms. Mining this rich, complex dataset with the goal of identifying ALS disease subtypes, requires sophisticated computational tools. We will develop a series of these tools, known as probabilistic graphical models (PGMs), to specifically interrogate and interpret the combined Answer ALS datasets. PGMs have been used extensively in applications from finance to artificial intelligence and healthcare diagnostics. These flexible, interpretable models are able to identify and quantify changes in cellular pathways. Creation and application of PGMs to enable integrated models of this comprehensive dataset, will enhance our understanding of disease mechanisms and advance the discovery of targets for effective therapy.

Baltimore - Johns Jopkins University

Jiou Wang Ph.D.

RG16   Novel Quality Control Pathways in ALS

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   We have found potential drug targets that need to be evaluated to determine their effectiveness in treating proteotoxicity-related neurodegeneration in ALS. The rationale for this proposal is based on the strong evidence of a tight link between ALS pathogenesis and protein quality control. Diverse forms of ALS are increasingly being connected with failed protein quality control in the nervous system. The research proposed here, if successful, will lead to new therapeutic agents that can help to treat both familial and sporadic patients of amyotrophic lateral sclerosis. Instead of targeting a specific disease protein, we hope to develop a "wide-spectrum" therapeutic strategy that could have a broad impact on the majority of ALS patients.

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	8/1/2017	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.

Bethesda - Federation of American Societies for Experimental Biology

Brent Ramsey MPA

CG   Skeletal Muscle Satellite Cells and Regeneration

$5,000.00	7/8/2018	7/13/2018	Year 1

Summary   "Skeletal Muscle Satellite Cells and Regeneration" is a part of the Federation of American Societies of Experimental Biology (FASEB) Science Research Conference series. Satellite cells (skeletal muscle stem cells) are the source of the remarkable regenerative properties of skeletal muscle, and their

study has major implications for understanding and treatment of muscle diseases and age-related muscle loss. As a relatively small, forward-looking meeting, this conference seeks to identify and explore areas of research that will be of future significance. The program includes speakers from around the world, reflecting the unusually collaborative and cohesive nature of the satellite cell community. Areas that will be covered during the conference include transcriptional, epigenetic, and metabolic regulation of satellite cells during homeostasis and regeneration, satellite cell quiescence and activation, satellite cell heterogeneity, alteration of satellite cells in aging and disease, and potential utility of satellite cells as therapeutics in muscle diseases. The conference will also have three dedicated poster sessions in the evenings, a Meet-the-Expert Session, and a Career-Oriented Workshop.

MASSACHUSETTS

Boston - Children's Hospital Boston

Alan H. Beggs Ph.D.

RG15   Molecular Genetics of Congenital Myopathies

$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.

Emanuela Gussoni Ph.D

RG16   Tetraspanin CD82 in dystrophic satelite cells

$99,253.00	2/1/2017	1/31/2018	Year 1
$98,937.00	2/1/2018	1/31/2019	Year 2
$99,795.00	2/1/2019	1/31/2020	Year 3

Summary   Dystrophic muscle stem cells are thought to be continuously active in an attempt to repair damaged myofiber due to lack of dystrophin and/or its associated proteins. We recently identified a novel cell surface marker for muscle stem cells in human and mouse muscle, named CD82. CD82 is expressed in human muscle satellite cells, both quiescent and activated. Importantly, CD82 protein expression is reduced in human DMD and in dystrophic mdx5cv mouse muscles. This application proposes to study the function of CD82 in muscle stem cells and understand how the decreased expression of this protein in dystrophic cells is linked to the disease progression. More importantly, the proposed studies will provide the groundwork for developing future therapies aimed at boosting expression of CD82 as a way to stabilize the sarcolemma and muscle stem cell function in dystrophic muscle.

Louis Kunkel Ph.D.

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

$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.

Angela Lek Ph.D.

DG16 Identifying genetic modifiers of FSHD

$60,000.00	8/1/2017	7/31/2018	Year 1
$60,000.00	8/1/2018	7/31/2019	Year 2
$60,000.00	8/1/2019	7/31/2020	Year 3

Summary FSHD is the most common autosomal dominant form of muscular dystrophy, yet we do not completely understand the genetics of the disease. Unlike most forms of muscular dystrophy, FSHD is unique and requires multiple genetic 'hits' in order to result in disease manifestation. Not all of these 'hits' have been resolved, which is why we do not understand why some relatives of FSHD patients that harbor the same permissive genetics are not affected. We propose to perform a genetic screen to identify these missing genetic links that appear to allow some individuals to be 'resistant' to FSHD. Using the latest in genome-editing technology, we will perform genetic modifications that result in systematically switching 'on' every gene, one by one, across the entire human genome. We hypothesize that one of these switches will result in reduction of FSHD-related cell toxicity, thus serving as a 'modifier' gene in FSHD that can potentially be used to ameliorate disease symptoms in patients. The candidate modifier genes we find will be cross-referenced to genomic sequencing data derived from FSHD patients and their asymptomatic carrier relatives, as a possible explanation for their clinical variance. We will further validate these genes with functional rescue experiments in our zebrafish model of FSHD, and also measure their ability to change the molecular disease signature of FSHD patient cells. Once validated, these target genes will serve as concrete targets for therapeutic development.

Boston - Dana-Farber Cancer Institute

Eduardo Balsa Martinez Ph.D

DG16 Combinatorial screens to identify novel targets to combat mitochondrial diseases

$60,000.00	2/1/2017	1/31/2018	Year 1
$60,000.00	2/1/2018	1/31/2019	Year 2
$60,000.00	2/1/2019	1/31/2020	Year 3

Summary Mitochondrial diseases are a heterogeneous class of conditions, mostly cause by mutations, that severely impair energy production. As a consequence, high-energy demanding tissues such as brain, heart and skeletal muscle are primary affected. Clinically, based on the severity of the cells and tissues affected, the symptoms are muscle weakness and pain/myopathies, loss of motor control, cardiac and liver diseases, seizures, visual/hearing problems, lactic acidosis and developmental problems. As it relates to treatments and therapies, at this time there are no cures for mitochondrial diseases. Thus, treatments are more often palliative and mainly include vitamin cofactors, nutritional manipulations, and exercise. Taken together the facts indicated above, this application proposes to investigate this medical urge by using a combination of genetic and chemical screens to identify novel targets to combat mitochondrial diseases. Interestingly, using this technology we identified an FDA-approved drug that is able to enhance the mitochondrial function in cells derived from patients carrying mitochondrial mutations. These cells are unable to generate enough energy to survived a die. Surprisingly, treatment with the discovered compound proved to be beneficial and energy production was partially restored, leading to cell survival. We aim to characterize the effects of this drug and further investigate whether it might be use in future therapies to combat mitochondrial disorders.

Boston - Harvard Medical School

Alfred L. Goldberg Ph.D.

RG16 Protein breakdown in muscle in normal and disease states

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

Summary   One major objective of our work is to clarify the molecular mechanisms for the muscle atrophy that occurs with congenital myopathies, neuronal disease (e.g. ALS), and many systemic diseases, in order to lay the basis for development of rational therapies. Our early work established that this debilitating loss of muscle mass is due primarily to excessive degradation of muscle proteins, and recently we identified key enzymes that mark muscle proteins for destruction during atrophy. In recent years, much has been learned about the proteasome, the molecular machine that degrades most cell proteins. We recently found that during atrophy the composition of proteasomes changes in ways that seem to contribute to the accelerated protein destruction. The proposed studies will explore the special features of proteasomes in atrophying muscle and their importance in disease states. Many muscle diseases are caused by the accumulation in muscles of aggregates of misfolded, mutated proteins, which normally are efficiently degraded by proteasomes. We recently found that the capacity of proteasomes to destroy such abnormal, toxic proteins can be enhanced by drugs that induce the phosphorylation of a key component of the proteasome. We will test whether this newly discovered method to activate the proteasomes might be useful in promoting the clearance of these toxic proteins and thus slow progression of these various diseases, for which, no effective treatment is known.

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

Nazem Atassi M.D.

HCTG   PET Imaging of Inflammation and Epigenetics in People with ALS

$363,836.00	12/1/2017	7/31/2018	Year 1
$368,100.00	8/1/2018	11/30/2019	Year 2

Summary   The two major limitations for developing effective treatments for people with ALS are: limited understanding of ALS causes and lack of tools to test promising treatments in patients. In order to overcome these limitations we need to study ALS in living patients and advanced imaging techniques provide a unique opportunity to study brain changes in patients. In this proposal we will build novel imaging tools to study ALS mechanisms and ultimately use these imaging technologies as readouts in future clinical trials. The proposed imaging tools will measure two main ALS mechanisms: inflammation and epigenetics. Our group built an imaging platform that showed increased inflammation in brain regions that are affected in ALS. In the first aim, we propose to build on our initial work and identify the timing of initial appearance of inflammation in people who carry one of the ALS genes but don't yet have ALS symptoms (Asymptomatic Gene Carriers). Identifying the time of initial brain biological changes will have a major impact on determining the time to start gene therapies in asymptomatic gene carriers. The second aim of this proposal is focused on testing a new imaging platform to measure DNA variations in the brains of people with ALS. Our imaging group developed a novel tracer called Martinostat that allows us to measure, in ALS patients, the same DNA changes we see in postmortem tissue.

Thurman Wheeler M.D.

RG16   Extracellular RNA as biomarkers of myotonic dystrophy

$110,000.00	2/1/2017	1/31/2018	Year 1
$110,000.00	2/1/2018	1/31/2019	Year 2
$110,000.00	2/1/2019	1/31/2020	Year 3

Summary   A new drug for treatment of myotonic dystrophy type 1 is being tested in clinical trials. Monitoring drug effects currently requires that patients undergo multiple muscle biopsies, a procedure that is invasive, painful, and, in pediatric patients, requires general anesthesia. The goal of this project is to develop biomarkers in human urine or blood that 1) will reduce or eliminate the need for muscle biopsies to determine whether treatments are working, 2) can be measured multiple times as needed during the trial, and 3) enable inclusion of children with myotonic dystrophy in upcoming trials. Our approach will be applicable to many different treatment strategies for both myotonic dystrophy types 1 and 2, and extend to other muscular dystrophies.

Boston - Northeast ALS Consortium

Jonathan D. Glass MD

RIG   Northeast ALS Consortium: Infrastructure support

$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.

Cambridge - Broad Institute, Inc.

Daniel MacArthur Ph.D

RIG — Scalable infrastructure for recruitment and whole-genome sequencing of muscle di

| $110,000.00 | 12/1/2017 | 11/30/2018 | Year 1 |

Summary — Approximately 80% of rare diseases are genetic, meaning that just one or two spelling changes in the genome are responsible for a patient's condition. Despite clinical care to find these mutations, over half of rare disease patients do not yet have a genetic diagnosis. Improving diagnostic rates will require integrating genomic data and clinical stories into studies. The low prevalence of similar rare muscle disease patients means that recruiting for a study with in-person visits is often infeasible. Working with MDA, we have developed the Rare Genomes Project to give patients the opportunity to participate in a research study no matter where they live. To participate, families visit raregenomes.org to describe their condition and provide consent. Sample kits are mailed to the family, and then returned for genome sequencing. If we identify the genetic cause of the condition, we will provide a diagnosis to the family and their doctor, in hopes of directly impacting clinical care.

Cambridge - Solid Biosciences, LLC

Kristy Brown Ph.D.

RG16 — Generating the Evidentiary Data Package for Dystrophin Biomarker Qualification

| $49,870.00 | 12/1/2017 | 11/30/2018 | Year 1 |
| $49,870.00 | 12/1/2018 | 11/30/2019 | Year 2 |

Summary — Duchenne muscular dystrophy patients are missing the muscle protein dystrophin. Therapies to restore this missing protein are currently being developed and tested. It is important to test if these drugs are working to get FDA approval. Because the drugs are restoring the missing dystrophin protein, detection of the new dystrophin is important. Additionally, it is also important to know exactly how much new dystrophin is being made. Currently we do not know how much new dystrophin is needed before the patient will improve or stabilize. The goal of this current project is to look at many patients with a wide range of dystrophin protein levels and determine that protein level with high accuracy. We will use already established muscle biopsy biobanks, so no new patient enrollment is needed. Next, we will compare the dystrophin level with the patient well-being to determine a relationship between dystrophin protein amount and the patient's over-all health. This study will help reveal the amount of new dystrophin needed to be made by these drugs to help patients. These data will be submitted to the FDA for review in their public Biomarker Qualification Program. Through this interaction with the FDA, all results will be made available to all researchers and patients to help all future drug programs that aim to treat Duchenne patients by restoring dystrophin protein. The overall goal is to help Duchenne patients and families by streamlining the clinical trial process.

Lexington - Izumi Biosciences Inc.

Antonius (Ton) Martinus Bunt M.D.

MVP15 — IZ10023 inhibits two efflux pumps and enhances riluzole efficacy in ALS

| $28,560.00 | 2/1/2017 | 2/1/2018 | Year 2 |
| $11,400.00 | 2/2/2018 | 7/1/2018 | Year 3 |

Summary — ALS is a rapidly progressive, lethal neurodegenerative disease where patients experience a transient benefit from riluzole, the only approved drug. In early stage, riluzole slows disease progression, whereas this benefit is lost in late stage. Two pumps (P-gp and BCRP) were over expressed locally in diseased spinal cords of ALS patients. We believe they collaborate to remove riluzole, cause ineffective local drug levels, and a loss of efficacy of riluzole. Elacridar is a potent, selective inhibitor of both pumps. Elacridar selectively targeted drug distribution to the pump-protected brain, with levels in plasma and peripheral organs relatively unchanged. In ALS mice, elacridar enhanced riluzole activity, and significantly prolonged survival. In six Phase I studies with 97 patients, oral elacridar was safe and well tolerated, yet did not reach required plasma levels. Izumi developed oral IZ10023 with greatly improved plasma levels. The 1.5 year project seeks to 1. test the effect of IZ10023 on riluzole brain levels in rodents, 2. manufacture drug supplies for animal and patient studies, 3. conduct FDA-

mandated safety studies in animals, 4. open an IND. In subsequent studies we seek to confirm that IZ10023 enhances riluzole efficacy while maintaining safety in ALS patients. The pump blocker IZ10023 may remove a key obstacle to effective drug therapy, and provide a foundation for future drug combinations to further improve the outcomes for ALS and possibly Alzheimer patients.

Waltham - PerkinElmer Genetics, Inc.

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

RG16   Precision medicine in muscular dystrophy: Variants of unknown signficance

$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.

Worcester - University of Massachusetts Medical School

Charles P. Emerson Ph.D.

RG16   iPSC-induced skeletal muscle progenitors to study human myogenesis and FSHD

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   Human induced pluripotent stem cells (hiPSCs) are produced by reprogramming adult cells (for example, skin cells) to become embryonic-like cells that can be turned into any cell type (i.e. are pluripotent) and grow indefinitely in tissue culture. The Emerson lab, together with their collaborators, have developed novel methods to induce hiPSCs to produce skeletal muscle cells that differentiate in cell culture and can be xeno-engrafted into mouse muscles where they differentiate into muscle fibers, allowing them to investigate how muscle diseases form and progress. We are studying facioscapulohumeral muscular dystrophy (FSHD), a disease of progressive muscle weakness, using hiPSC models. FSHD patient-derived hiPSCs induced to skeletal muscle express the FSHD disease gene, DUX4, and other biomarkers, both in cell culture and xenograft muscle, establishing that our hiPSC myogenesis system can model FSHD molecular pathology. We propose to utilize hiPSC myogenesis to investigate FSHD disease mechanisms in muscle cell and engraftment models using hiPSCs from FSHD patients with infantile/early onset, adult onset, and non-manifesting disease. Our findings will inform us about the contributions of FSHD disease genes and epigenetic dysregulation to FSHD clinical disease severity and muscle pathology and identify new drug targets for therapeutics.

Fen-Biao Gao Ph.D. 1995

RG16   Investigation of DNA damage as a therapeutic target in C9ORF72-related ALS

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   C9ORF72 repeat expansion is the most common genetic mutation in ALS. To understand its pathogenic mechanisms, we generated induced pluripotent stem cells (iPSCs) from C9ORF72 patients and differentiated them into motor neurons and cortical neurons that recapitulate some key neuropathological features, such as abnormal accumulation of RNA "clumps" and production of unusual toxic protein species (Almeida et al., Acta Neuropathol. 2013; López-González et al., in preparation). To dissect the pathogenicity of these disease molecules, we used fruit fly Drosophila and provided some important insights (Tran et al., Neuron 2015; Freibaum*, Lu* et al., Nature 2015; Yang et al., Acta Neuropathol. 2015). In this proposal, we will continue to use C9ORF72 patients' iPSCs-derived motor neurons as our experimental system together with some studies in fruit flies. We will build upon a large body of preliminary results we have already obtained, such as that iPSC-derived C9ORF72 motor neurons showed an age-dependent increase in the number of detrimental damages to

the genome. These studies will greatly enhance our understanding of ALS pathogenic mechanisms and may identify components in the DNA damage pathway as potential therapeutic targets.

Mahasweta Girgenrath Ph.D

RG15	Utilizing natural history to identify optimal timeline for combinatorial therapy		
$100,000.00	2/1/2017	7/31/2018	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.

MICHIGAN

Ann Arbor - The Regents of the University of Michigan

Asim Beg Ph.D.

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

Andrew Lieberman M.D., Ph.D.

RG16	Modified antisense oligonucleotides to treat spinobulbar muscular atrophy		
$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   Spinal and bulbar muscular atrophy (SBMA) is a degenerative disorder of motor nerve cells and skeletal muscle caused by a mutation in the androgen receptor, the protein that binds male sex hormones. The disease causes progressive muscle weakness only in men, and no therapies are currently available. The objective of this application is to complete preclinical studies in a mouse model to establish the safety and efficacy of a new type of therapy to silence expression of the mutant gene. We will deliver this drug under the skin and target skeletal muscle; in fact, this new drug has been specially designed for efficient uptake by muscle. Our central hypothesis is that the enhanced targeting of muscle by this new drug will enable robust gene silencing at lower doses, thereby limiting off target toxicity while concurrently enhancing activity in a variety of disease-relevant muscles. These studies build upon our prior data pointing to significant benefits from this type of approach, and leverage the recent discovery of a chemical modification that increases drug uptake by skeletal muscle ~3-5-fold. We will use genetic, biochemical, histological and behavioral analyses to accomplish these

goals. We will (1) establish the extent to which this modified drug triggers enhanced gene silencing and prevents disease onset in SBMA mice, and (2) determine effects of this drug in symptomatic SBMA mice. These studies are expected to provide essential efficacy data in a preclinical model.

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   8/1/2017   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.

Nam Chul Kim Ph.D.

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

$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.

Michael Kyba PhD

RG   Determinants of self-renewal and differentiation of satellite cells

$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.

Joseph M Metzger Ph.D

RG16   Molecular basis of dystrophic cardiomyopathy

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/30/2020	Year 3

Summary   The MDA has the ultimate goal of finding a cure for DMD. Despite great efforts there is no cure for DMD. In this context, it is reasonable to propose that an effective treatment that could prevent or even delay key aspects of DMD muscle disease would be the next best outcome in place of a cure. Ongoing genetic-based therapies, such as exon skipping and gene therapy, are advancing through clinical trials. These therapies involve a treatment in which a shortened dystrophin molecule is made by "deleting" regions of the gene. We have found, unexpectedly, that truncated dystrophin molecules can have markedly reduced stability in striated muscle in vivo as compared with normal full length dystrophin. We propose here a high precision truncated dystrophin peptide "titration" platform to directly access the in vivo stability of clinically relevant shortened dystrophin proteins in striated muscle. Outcome of this work is essential to define the key parameters of dystrophin dosing and function required for the ultimate success of on-going and future genetic-based clinical trials for DMD.

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

RG   Hypoxia as a Modulator of Dystrophic Cardiomyopathy

| $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 | 2/1/2017 | 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

Aaron DiAntonio M.D.,Ph.D.

RG   Mechanism of axon loss in congenital motor neuropathies

| $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.

Timothy M Miller M.D.,Ph.D

RG16   Understanding RAN Dipeptide Size and Kinetics in c9orf72 ALS

$94,287.00	8/1/2017	7/31/2018	Year 1
$94,060.00	8/1/2018	7/31/2019	Year 2
$94,070.00	8/1/2019	7/31/2020	Year 3

Summary   The most common genetic cause of ALS is a hexanucleotide repeat expansion mutation in the c9orf72 gene, causing 30% of familial ALS and 5-10% of non-inherited ALS cases. This repeat expansion causes the accumulation of dipeptide repeat proteins (DPRs), which aggregate in human tissues and have been found to be toxic in cellular and animal models. However, pathological characteristics of the DPRs do not seem to correlate with clinical characteristics or degenerative severity in patients, making it challenging to determine whether these DPRs directly contribute to degeneration in human ALS. Thus, while DPRs are an attractive therapeutic target, there remains a missing link between promising cell/animal work and human disease. In this grant, we propose to further investigate aspects of DPRs to determine their significance to human disease using innovative methodologies. We will develop a novel way to understand DPR size in human tissues and cerebral spinal fluid (CSF) as well as a method to determine the turnover rate of DPRs in CSF of c9orf72 expansion carriers. The turnover rate of DPRs may correlate with disease measures and could help define when and how to apply therapeutics. We have successfully developed methods for kinetic analysis of other proteins such as SOD1 in ALS and tau in Alzheimer's disease. We are well-prepared to carry out these studies, which have important implications in the understanding and treatment of c9orf72 ALS.

Daniel Summers Ph.D

DG   Molecular Mechanisms of NAD+ Homeostasis in Peripheral Neurons

$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	8/1/2017	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.

NEVADA

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

Dean J. Burkin Ph.D.

RG16   Alpha7 integrin enhancing small molecule for Duchenne Muscular Dystrophy

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   Duchenne muscular dystrophy (DMD) is a fatal muscle disease for which there is no cure and limited treatment options. DMD is caused by mutations in the gene dystrophin that results in a complete absence of the dystrophin protein. Dystrophin forms a scaffold which serves as a molecular glue that binds muscle cells together and transmits force during muscle contraction. Loss of dystrophin causes muscle fibers to be damaged which causes muscle weakness. A second molecular glue called the alpha7beta1 integrin is also present in muscle and studies have shown that increased levels of this second system can prevent disease progression. We have conducted a drug screen and identified a compound that increases the alpha7beta1 linkage system in muscle. This compound has an FDA approved analog and in this proposal we aim to test this in mouse models of DMD. Since this is already an FDA approved drug, a successful outcome of this project may more rapidly translate this as a new integrin-based therapy for DMD patients.

Peter Jones PhD

RG15   FSHD-like mice for therapeutic development and preclinical testing

$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.

NEW JERSEY

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	8/1/2017	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

Binghamton - The Research Foundation of SUNY at Binghamton University

Yetrib Hathout Ph.D.

RG — Development and validation of pharmacodynamic biomarkers for Duchenne.

| $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.

New York - Columbia University Medical Center

Hiroshi Mitsumoto M.D.

RRG2 — 2016 WOW - Additional analyses of the ARREST ALS and ALS COSMOS studies in ALS

| $100,950.81 | 1/1/2018 | 12/31/2018 | Year 1 |

Summary   Since its inception in 2011, MDA Wings Over Wall Street (MDA Wings) funding has provided incredible support for basic and clinical research in ALS conducted at our MDA/ALS Center at Columbia University Medical Center. For the past several years, MDA Wings has supplemented our large NIEHS- and CDC-funded clinical and epidemiological studies. This year, we request support for a few additional projects to determine 1. if data on patients from the ALS COSMOS and ARREST ALS projects are comparable in order to combine datasets and obtain more impactful results, 2. if internet-based self-report (National ALS Registry) and interview-based data (ARREST ALS project) are comparable for the analyses of environmental risk factors. We can use either internet-based or structured interview-based methods to obtain data on environmental risk factors. The third project is to continue the analyses of the ALS COSMOS study to determine the relationship between oxidative stress and disease progression in ALS. We will explore if the degree of physical exercise affects disease progression and if there are any differences in various risk factors (cognitive, psychological, and environmental) as well biomarkers among clinically separated ALS subsets (ALS, PMA, and PLS). We hope our study will generate high-impact results and will improve our understanding of disease mechanisms of amyotrophic lateral sclerosis.

Jacqueline Montes EdD

CTTG — Evaluation of Oxidative Capacity and Exercise Tolerance in Ambulatory SMA

$23,520.00	4/1/2018	3/31/2019	Year 1
$23,520.00	4/1/2019	3/31/2020	Year 2
$20,580.00	4/1/2020	5/1/2021	Year 3

Summary   This project will examine the pathophysiological underpinnings of reduced exercise capacity in patients with Spinal Muscular Atrophy (SMA). Ambulatory SMA patients have a marked reduction in oxidative capacity and blunted conditioning response to exercise. In contrast, other neuromuscular conditions

derive significant benefit from aerobic conditioning despite having similar clinical presentations and functional limitations. There has been laboratory evidence to suggest that the molecular mechanisms underlying mitochondrial biogenesis may be vulnerable to SMN deficiency. A reduction in oxidative capacity disproportionate to lean mass and disease severity would further support evidence of mitochondrial depletion in SMA. Alternative exercise training strategies and/ or concomitant targeted therapeutic intervention may be necessary to achieve an aerobic conditioning effect. Understanding potential differences in composition and oxidative capacity among leg muscle groups will permit directed exercise training paradigms exploiting muscle groups most amenable to elicit a training effect. This study will (1) estimate oxidative capacity of specific muscle groups during exercise using near infrared spectroscopy and (2) describe body composition to better understand exercise capacity and mitochondrial function in ambulatory SMA patients and disease controls. It is a 6-month observational study including 14 ambulatory SMA, 14 ambulatory mitochondrial myopathy, and 14 healthy controls.

Liza Pon Ph.D.

RG | A newly identified congenital muscular dystrophy: mechanisms and interventions
$100,000.00	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.

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	8/1/2017	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	2/1/2017	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 - Johns Hopkins University

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

RG15	Modeling Duchenne muscular dystrophy with hiPSCs and pharmacological rescue			
	$100,000.00	2/1/2017	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.

New York - Target ALS

Manish Raisinghani

RIG	Target ALS Biomarker Consortium			
	$100,000.00	12/1/2017	8/1/2018	Year 1

Summary

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

James Manley Ph.D

RG15	Senataxin, mutated in ALS4, regulates autophagy			
	$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

Douglas Anderson Ph.D.

DG15	Mitigating Muscular Dystrophy with a Calcium-regulatory Micropeptide			
	$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.

Johanna Hamel M.D.

CRTG   Comparative Studies of RNA Toxicity in Myotonic Dystrophy

$43,333.00	7/1/2017	6/30/2018	Year 1
$43,333.00	7/1/2018	9/30/2019	Year 2

Summary

Johanna Hamel M.D.

CG   Neurotherapeutics Symposium 2018

$7,500.00	12/1/2017	1/28/2018	Year 1

Summary

Neurotherapeutics Symposium 2018, to be held in Orlando, Florida, February 7-9 2018, focuses on development of experimental therapeutics (testing new drugs to help neurologic diseases). It will give updates on the current research developments in neuromuscular diseases and movement disorders and offer a platform for young investigators of diverse ethnic backgrounds to establish or deepen mentoring relationships with advanced investigators. It is open to clinicians, researchers, and trainees interested in neurotherapeutics. It brings together experts studying different diseases and subspecialties for a fruitful exchange of ideas. A major emphasis is on stimulating young neurologists of diverse backgrounds to develop a career in experimental therapeutics. The first session includes talks covering pathomechanisms of disease and possible treatment targets, outcome measures, updates in current clinical trials, and the process of drug approval. The second part contains interactive sessions on clinical considerations (adopting new drugs into practice and monitoring for treatment effects and side effects) with discussions between scientists involved in drug development and clinical trials and physician scientists or clinicians involved in patient care. The third part is a dedicated young investigator session. Participants will have the opportunity for an informal exchange of research ideas and advice on career development with junior and senior investigators of diverse backgrounds.

Charles Thornton MD

RRG2   2016 Emilio Genzano Fund - Muscle Transcriptome in DM1 Model

$44,610.00	4/1/2018	3/31/2019	Year 1

Summary

The goal of this research is to speed up the process of developing an effective treatment for myotonic dystrophy type 1, by creating a good system for testing new medications before they are given to people.

Charles Thornton MD

CRNG   Myotonic Dystrophy Clinical Research Network

$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.

Stony Brook - Research Foundation of the State University of New York

Aaron M Beedle Ph.D.

RG16   Is mTOR inhibition a valid therapy for dystroglycan muscular dystrophy?

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   Dystroglycan-related muscular dystrophies are caused by abnormal processing of the protein alpha-dystroglycan. The abnormal processing impairs alpha-dystroglycan function causing muscular dystrophy with variable involvement of the heart, brain and eyes. Dystroglycan-muscular dystrophies are caused by mutations in many different genes, making it difficult to develop gene therapy strategies because there are so many gene targets. Instead, this goal of this proposal is to test a pharmacological approach inhibiting a common intracellular pathway involving a protein called mTOR. Preclinical studies will be conducted to test the benefit of an mTOR inhibitor for improving muscle function, reducing pathology, and extending lifespan, while monitoring for potential drug adverse effects in mice with dystroglycan muscular dystrophy. A significant therapeutic benefit in these studies has the potential for rapid translation to the clinic.

NORTH CAROLINA

Charlotte - Carolinas HealthCare Foundation

Benjamin Rix Brooks M.D.

RRG2   2016 Bojangles Golf Classic - Pentose Alcohol Therapy for Limb Girdle 2i - Clinical Trial Preparation

$283,205.00	3/1/2018	2/28/2019	Year 1

Summary   Mutations in the Fukutin Related Protein (FKRP) produce a wide range of phenotypes from Limb Girdle Muscular Dystrophy 2i (LGMD2i) to congenital muscular dystrophy (CMD) to Walker Warburg Syndrome (WWS). These membrane based myopathies have been resistant to treatment. The observation by the McColl-Lockwood Laboratory for Muscular Dystrophy Research is that a pentose alcohol, ribitol, may correct some of the metabolic abnormalities in cells, and pre-clinical models of these diseases compels moving forward with translation of this treatment for people with these conditions. Developing a treatment for people requires careful assessment of potential clinical toxicity of the treatment in animals before the introduction of the treatment to humans in early phase safety and tolerability clinical trials. The studies outlined in this proposal include completing animal toxicity studies and dose ranging studies for efficacy in pre-clinical animal models. In parallel, patients with known FKRP mutations will be identified and characterized for preliminary human safety, tolerability and dose ranging clinical trials. Novel home-based clinical measures of activity will be compared with standard clinic-based outcome measures to allow large numbers of patients with these rare conditions to partake in the development of treatments globally.

Durham - Duke University Medical Center

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

RG16   SBMA motor neuron degeneration: From cellular and molecular basis to therapy

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   X-linked spinal & bulbar muscular atrophy (SBMA) is an inherited neuromuscular disorder characterized by lower (spinal cord) motor neuron degeneration. SBMA is caused by CAG/polyglutamine repeat expansions in the androgen receptor gene. My research has focused on defining the cellular and molecular basis of SBMA by using a variety of approaches, including neurons grown in culture, genetically engineered mice, and "stem cell" models derived from SBMA patient skin cells by reprogramming these skin cells to become pluripotent stem cells which we then differentiate into immature neurons, motor neurons, and skeletal muscle. Our published work over the last two years has yielded two important findings for SBMA disease research, with implications for understanding more common motor neuron diseases, including spinal muscular atrophy (SMA) and

amyotrophic lateral sclerosis (ALS). We discovered that expression of mutant AR protein in skeletal muscle is required for SBMA motor neuron disease, and now wish to understand how skeletal muscle dysfunction sickens motor neurons. What we learn may provide targets for therapy development for not only SBMA, but also SMA and ALS. We identified dysfunction of a factor known as TFEB as the cause of SBMA defects in protein quality control pathways, and now propose to determine why this occurs, again expecting that an understanding of this disease mechanism will reveal targets for therapy development for SBMA and related motor neuron diseases.

OHIO

Columbus - Research Institute at Nationwide Children's Hospital

Scott Q. Harper Ph.D.

RG16   Therapeutic development for FSHD using a new DUX4-expressing knockin mouse

$100,000.00	2/1/2017	1/31/2018	Year 1
$100,000.00	2/1/2018	1/31/2019	Year 2
$100,000.00	2/1/2019	1/31/2020	Year 3

Summary   No treatment exists for Facioscapulohumeral muscular dystrophy (FSHD). FSHD is caused by expression of the toxic DUX4 gene in muscle, and so strategies for treatments should focus on reducing or turning off DUX4. Before new treatments can be used in humans, they must first be tested in animal models to ensure that they (1) work and (2) are safe. In the case of FSHD, this would ideally mean having an animal model that expresses DUX4 and has diseased muscles, which could then be treated with an anti-DUX4 therapy. Although mice have been created that contain the DUX4 gene in their chromosomes, they unfortunately do not develop muscle disease or they die before birth. This fact represents a roadblock for therapy development. We have recently developed such a mouse, and are seeking funding to characterize it and use it for DUX4 inhibition therapy testing.

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	2/1/2017	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

$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	8/1/2017	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 - The Children's Hospital of Philadelphia

David Lynch MD, PhD

RG16   Synaptic dysfunction in Friedreich Ataxia

$100,000.00	2/1/2018	1/31/2019	Year 1
$100,000.00	2/1/2019	1/31/2020	Year 2
$100,000.00	2/1/2020	1/31/2021	Year 3

Summary   Friedreich Ataxia (FRDA) is the most common form of inherited ataxia and a fatal neurodegenerative disease with onset in early childhood, affecting 1 in every 50,000 people in the United States. The mean age at death is 35 related to coexistent heart disease. Symptoms begin as early as 5 years old and worsen over time, with major neurological symptoms being balance difficulty and incoordination. FRDA is caused by deficiency of the mitochondrial protein frataxin for which there is no cure or effective treatment. A variety of clinical trials in FRDA have shown an unexpected short term response to mitochondrial enhancers (A0001/Idebenone) and frataxin restoration drugs (interferon gamma) particularly in speech dysfunction and fatigue. However, speech dysfunction in FRDA is not mediated by brain regions in which cell loss occurs early in the disease; this time course is also inconsistent with cell death. Consequently, the findings observed in our preliminary data on disrupted synaptic connections between cerebellar neurons in an FRDA mouse model match unexplained findings from clinical trials in FRDA patients. The present proposal will determine if rescuing mitochondrial biogenesis or synaptic deficits can reverse cerebellar synaptic dysfunction and ataxic phenotypes in the FRDA mouse models. If successful, our findings will improve understanding of neurological dysfunction in this disease, which can immediately translate to novel avenues for treatment of FRDA patients.

Philadelphia - The Trustees of the University of Pennsylvania

Tejvir S. Khurana MD, Ph.D.

RG16   Utrophin upregulation via let-7c SBO-mediated miRNA repression for DMD therapy

$80,000.00	2/1/2017	1/31/2018	Year 1
$80,000.00	2/1/2018	1/31/2019	Year 2
$80,000.00	2/1/2019	1/31/2020	Year 3

Summary   Utrophin is highly related to the dystrophin gene. It is of great therapeutic interest since increasing its production in muscles can compensate for the lack of dystrophin in animal models of DMD. We have found that utrophin is in a state of repression and that a class of molecules called microRNA's (miRNAs) cause the repression. We will develop site-blocking oligo- based methods to repress the micro RNA let-7c repressor, in order to achieve Utrophin upregulation. These approaches will be tested in the mdx mouse model of DMD.

Philadelphia - Thomas Jefferson University

Angelo C Lepore Ph.D.

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

$100,000.00   8/1/2017   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   8/1/2017   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   8/1/2017   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	11/1/2017	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.

Christi L Kolarcik Ph.D.

DG16   Motor system connectivity influences in amyotrophic lateral sclerosis

$60,000.00	8/1/2017	7/31/2018	Year 2
$60,000.00	8/1/2018	7/31/2019	Year 3

Summary   Communication between neurons and muscles occurs at synaptic connections which are often compromised in neuromuscular disease. Multiple hypotheses on where connections breakdown exist for amyotrophic lateral sclerosis (ALS). Do the earliest changes occur at the spinal motor neuron or the cortical level? How do disruptions in axonal transport contribute? Underlying these potential mechanisms is the neural circuitry which ultimately mediates the pathological process. This proposal will unravel the synaptic connections to motor neurons innervating affected muscles and determine how these change in ALS. Our hypothesis is that synaptic inputs to muscles affected in ALS will change with disease progression; these inputs will enable us to predict disease spread through the motor system. First, we will map the neural circuitry that controls the motor neurons innervating two hindlimb muscles in the mouse using transneuronal viral tracers. Both fast- and slow-twitch muscles will be included to identify muscle fiber/motor neuron-type specific changes. By defining the direct and more elaborate multi-synaptic pathways, we can determine how and when synaptic connections are affected as ALS progresses. We will investigate pre-symptomatic, denervation, symptom onset and end-stage disease phases. The time course of synaptic connectivity changes and transport deficits will be elucidated, providing insights into mechanisms underlying degeneration and leading to targeted therapeutic options.

Udai Bhan Pandey Ph.D.

RG16   Identification of therapeutic small moleules for FUS-specific ALS

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   ALS is a devastating motor neuron disease for which currently no effective therapies available. We propose to identify drugs that can suppress symptoms associated with ALS.

Araya Puwanant M.D.

RG16   Sub-regional Body Composition and Clinical Endpoints in Myotonic Dystrophy

$99,719.00	2/1/2017	1/31/2018	Year 1
$99,387.00	2/1/2018	1/31/2019	Year 2
$99,746.00	2/1/2019	1/31/2020	Year 3

Summary   Myotonic dystrophy is the most prevalent form of muscular dystrophy in adults, characterized by progressive muscle weakness, muscle stiffness, and multi-organ involvement. This condition causes progressive disability and significant burden for affected individuals and caregivers. In recent years there has been significant progress in developing specific treatment for this condition. This project will facilitate therapeutic development by identifying practical and reliable endpoints for progressive muscle weakness and wasting in patients with myotonic dystrophy.

RHODE ISLAND

Providence - Brown University

Kristi A. Wharton PhD

RG16   Suppression of ALS-associated degeneration

$103,750.00	1/1/2018	6/30/2018	Year 1

Summary   Amyotrophic lateral sclerosis (ALS) is a disease that destroys the function of our motor neurons, resulting in their degeneration and ultimate death. The loss of motor neurons results in a failure of our muscles to function properly, such that walking, running, talking, chewing and breathing become uncoordinated and severely impaired. The exact cause of ALS remains elusive, in fact a number of different genetic mutations have been identified in ALS patients and a number of different cellular processes are implicated in generating motor neuron loss, suggesting that the disease is likely to arise from defects in multiple processes. Thus, it is likely that success in identifying a therapeutic intervention(s) as well as a cure, will only come when the ability to suppress ALS-associated degeneration is evaluated in the context of the whole organism with an intact nervous system and motor circuit. In our collaboration with Pfizer, we intend to use ALS models in genetically tractable organisms, Drosophila and C. elegans, to perform both chemical and genetic screens for factors that alleviate degeneration. In this application we will optimize screening design and establish feasibility for screens of the Pfizer chemogenomics library using well characterized Sod1-ALS models for which we know suppression is possible.

TEXAS

Dallas - UT Southwestern Medical Center

Ronald Haller M.D.

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

$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	2/1/2017	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.

Amy Hanna Ph.D.

DG16   Calsequestrin and ER Stress in muscle myopathy

$60,000.00	2/1/2018	1/31/2019	Year 1
$60,000.00	2/1/2019	1/31/2020	Year 2
$60,000.00	2/1/2020	1/31/2021	Year 3

Summary   Muscle contraction is a carefully controlled process that relies on the release of calcium from a storage compartment inside the muscle fiber. Calcium release is controlled by a protein called the ryanodine receptor (RyR1). Mutations in the ryanodine receptor cause skeletal muscle disease, one of the most common being central core disease (CCD). CCD causes delayed development in infants and severe muscle weakness in adults. Patients are also more likely to have skeletal abnormalities and to suffer from other ryanodine receptor linked diseases like malignant hyperthermia. There are currently no treatment options for CCD. One potential cause of myopathy in CCD is the accumulation of misfolded protein inside the muscle fiber. However, it is unknown how a mutation in the ryanodine receptor leads to accumulation of misfolded proteins. The current proposal is based on new evidence that

supports a role for calsequestrin, a protein that regulates the ryanodine receptor. We hypothesize that calsequestrin is redistributed in CCD muscles, triggering the activation of signaling pathways that cause muscle myopathy. This proposal will use a new animal model of calsequestrin-linked myopathy to directly test if abnormal calsequestrin can trigger stress pathways in muscle. Understanding the pathways that lead to muscle myopathy will be crucial in the development of new therapeutic options that restore muscle size and strength and improve the quality of life for CCD patients.

James Lupski M.D., Ph.D., D.Sc. (hon)

RG16   Enhancing molecular diagnostics for unsolved neuromuscular disease cases.

$100,000.00	8/1/2017	7/31/2018	Year 1
$100,000.00	8/1/2018	7/31/2019	Year 2
$100,000.00	8/1/2019	7/31/2020	Year 3

Summary   Determining genetic diagnoses in neuromuscular diseases (NMDs) are particularly difficult due to the large number of disease-causing genes and significant overlap in clinical symptoms between patients. Our proposal aims to advance molecular diagnostics for NMD patients that remain undiagnosed after clinical diagnostic whole exome sequencing (WES) and enhance discovery of novel disease genes. We aim to first comprehensively re-analyze clinical exome data in a research setting to confirm that a diagnosis has not been missed. Our next aim is to perform exome sequencing for the patient's parents and/or other family members. This will help us find spontaneous or de novo mutations and novel genes associated with NMDs. For cases that remain unsolved, we will then carry out RNA sequencing from affected tissue, if available. The advantage of RNA sequencing from muscle tissue is that it can provide direct evidence of the functional impact of a mutation. We also propose whole genome sequencing (WGS) for cases where tissue is not available, as with decreasing sequencing costs, it is important to test whether WGS should replace WES in the clinic. Obtaining a molecular diagnosis is key for patients and families with NMDs as it affects family planning decisions, disease management, and participation in disease-specific registries and clinical trials.

Lubbock - Texas Tech University Health Sciences Center

Laxman D. Gangwani Ph.D.

RG16   Pharmacological inhibition of JNK for the treatment of spinal muscular atrophy

$93,500.00	2/1/2017	1/31/2018	Year 1
$93,500.00	2/1/2018	1/31/2019	Year 2
$93,500.00	2/1/2019	1/31/2020	Year 3

Summary   Spinal muscular atrophy (SMA) is caused by the low levels of the survival motor neuron (SMN) protein and is characterized by degeneration of spinal motor neurons. Reduction in degeneration of neurons has been shown to slow the progression of SMA disease. Treatments to prevent or slow neurodegeneration are unavailable. Recently, we have shown that the c-Jun NH2-terminal kinase (JNK) pathway mediates neurodegeneration in SMA. The neuron-specific isoform JNK3 is required for neurodegeneration in SMA. We have validated JNK3 as a therapeutic target using SMA mice. Genetic inhibition of JNK in vivo by knockout of the Jnk3 gene results in improvement of SMA phenotype. JNK3-deficiency provides neuroprotection, reduces muscle degeneration, improve muscle growth, motor function and overall growth and increases lifespan of SMA mice that shows a systemic improvement of SMA phenotype. We propose to test the pharmacological inhibition of JNK for the treatment of SMA using JNK inhibitors in SMA mouse model.

UTAH

Salt Lake City - University of Utah

Nicholas Johnson M.D.

HCTG   Trial Readiness and Endpoint Assessment in Congenital Myotonic Dystrophy

$199,539.00	12/2/2017	12/1/2018	Year 2
$198,909.00	12/2/2018	12/1/2019	Year 3

Summary   Congenital myotonic dystrophy is the most severe form of myotonic dystrophy type-1. This pediatric disorder causes severe disability throughout childhood. Currently, there are no available treatments for congenital myotonic dystrophy. In this project, we will develop meaningful clinical endpoints in congenital myotonic dystrophy to allow these children to participate in potential clinical trials. Children with congenital myotonic dystrophy will have measurements of strength, cognition, and quality of life

measured over the course of years. Measurements of the changes within the muscle will be used to see how the disease progresses over time. We will find the measurements that would be best used in a clinical trial. This is a necessary step to taking developing therapies into this patient population. The long-term goal is to reduce the disability for children with this disease.

VIRGINIA

Richmond - Virginia Commonwealth University

Montserrat Samso Ph.D

RG   Structural Analysis of RyR1 with Central Core Disease Mutations

| $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

Joel R. Chamberlain Ph.D.

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

| $100,000.00 | 8/1/2017 | 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.

Donghoon Lee Ph.D.

RG   Magnetic Resonance Biomarkers for Effective Treatments for Muscular Dystrophy

| $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.