

**ACTIVE GRANTS DURING
CALENDAR YEARS: 2010,2011,2012,2013**

FUNDING PROGRAM LEGEND

(CNRG)	Clinical Research Network Grant
(RRG)	Restricted Research Grant
(RF)	Research Fellowship
(RG)	Research Grant
(SG)	Special Grant
(DG)	Research Development Grant
(TCL)	Tall Cedars of Lebanon
(CRTG)	Clinical Research Training Grant
(TRAC)	Translational Research Grant
(TRIND)	Translational Research IND Planning Grant
(TRIG)	Infrastructure Grant
(MVP)	MDA Venture Philanthropy Grant

AUSTRALIA

Clayton - Monash University

Peter Currie Ph.D.

(RG) Elucidation of cellular pathology in muscular dystrophy, using zebrafish models

\$115,710 1/1/2010 12/31/2010 Year 3

Summary We propose to use fluorescent transgenic technology to follow individual detached fibers to determine what happens to cell when they detach and use different treatments to prevent the death of muscle cells in these models. Furthermore, we are now able to test the extent to which muscle degeneration in both models is a direct result of perturbation of the dystrophin-glycoprotein complex (DGC), alterations of which cause the vast majority of muscular dystrophy in humans.

Christina Anne Mitchell Ph.D.

(RG) Role of FHL1 in regulating skeletal muscle mass and myopathy

\$104,320 7/1/2009 6/30/2010 Year 2

\$110,796 7/1/2010 6/30/2011 Year 3

Summary Factors that promote muscle growth (hypertrophy) are potential new treatments to reduce muscle wasting (atrophy). We have investigated a protein called FHL1, and predict FHL1 controls muscle hypertrophy and wasting. We have engineered mice to produce excess FHL1. These mice exhibit increased muscle size, greater muscle strength and are protected from age-induced weakness. We have shown FHL1 stimulates hypertrophy by regulating two known factors that control muscle mass, NFATc1 and Foxo. Very recently, multiple errors, called mutations, have been identified in the FHL1 gene in three human myopathies. The effect of mutant FHL1 on muscle growth versus wasting will be determined by engineering mutant FHL1 expression in muscle cells, and measuring NFATc1 and Foxo activity, characterizing the molecular basis of these interactions. Duchenne Muscular Dystrophy (DMD) is caused by loss of the protein dystrophin. The protein utrophin can substitute for dystrophin and is a potential treatment for DMD. Utrophin

expression is stimulated by NFATc1. Given that FHL1 increases NFATc1 activity, we will investigate if FHL1 can also stimulate production of utrophin in muscle cell lines and in a mouse model of DMD. We will also examine if FHL1 can reduce muscle wasting in a mouse model of this disease.

Concord - Anzac Health & Medical Research Foundation

Garth A. Nicholson M.D., Ph.D.

(RG) Analysis of structural and regulatory elements of CMTX3 candidate genes

\$86,493	1/1/2010	12/31/2010	Year 1
\$86,493	1/1/2011	12/31/2011	Year 2

Summary Our goal is to explore new mechanisms of axonal degeneration by identifying the gene causing an X-linked form of Charcot Marie Tooth (CMTX3) Neuropathy. Using bioinformatic resources and next generation deep sequencing we will examine the entire genomic structure (introns and splice elements) and regulatory elements of the known candidate genes in the CMTX3 interval. In addition, the 2.5 million base pairs spanning the CMTX3 region will be examined for copy number variation (CNV) using a targeted microarray strategy. These experimental approaches will provide a systematic screen for identifying structural and regulatory mutations as a cause for CMTX3. Discovery of this gene mutation will provide a means to determine mechanisms causing axonal degeneration and therapeutic treatment strategies.

Crawley - The University of Western Australia

Steve D. Wilton Ph.D.

(RG) Refined AO design for enhanced dystrophin exon skipping

\$110,352	7/1/2009	6/30/2010	Year 3
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Summary Investigators will refine AOs for exon skipping therapy development for DMD

Steve D. Wilton Ph.D.

(RG) Preclinical assessment of splice switching oligomers

\$122,700	7/1/2010	6/30/2011	Year 1
\$122,700	7/1/2011	6/30/2012	Year 2
\$122,700	7/1/2012	6/30/2013	Year 3

Summary We have developed genetic bandaids (oligomers) to induce exon skipping to by-pass DMD-causing mutations. From a concept first demonstrated in vitro, then in animal models and first proof of concept studies in man, clinical trials are underway to evaluate two band aid types (2OMe and PMO). Another bandaid type (MOE) has a proven safety record, with clinical application for more than 12 months in over 1000 individuals. MOE bandaids appear well suited for exon skipping, but until now these oligomers have not been available for study. We will undertake detailed pre-clinical studies in animal models to assess their suitability for exon skipping. We have developed scores of AOs to by-pass other amenable dystrophin mutations. An often-cited and valid concern regards potential off-target effects, due to either the bandaid chemistry or non-specific binding. Could splice switching dystrophin oligomers anneal to similar RNA sequences and subsequently influence their expression? This project will detect potential cross-reactions between our optimized dystrophin bandaids and other gene sequences. Experiments will confirm predicted expression changes as well as variation associated with the

nature of the bandaid types used. If these compounds are shown to be very specific, this would confer greater confidence that non-specific effects will be minor. If some bandaids caused adverse events, this data could be used to develop strategies to minimize or by-pass the problem.

Melbourne - Baker IDI Heart and Diabetes Institute

Paul Gregorevic Ph.D.

(RG) Gene-transfer based follistatin expression for treatment of muscular dystrophy

\$104,500 1/1/2010 12/31/2010 Year 3

Summary Restoring dystrophin expression in patients' muscles early in life may halt further strength loss, but patients often already display considerable muscle weakness when diagnosed. Co-delivering genes that stimulate growth of muscle fibers may help to compensate for the loss of muscle strength seen in severe muscular dystrophies. I have observed that follistatin genes delivered in particles made with viral proteins can dramatically increase the strength of muscles in healthy mice, and when delivered with a dystrophin gene can improve the strength of a single muscle in dystrophic mice more than treatment with the dystrophin gene alone. I will assess if delivery of a follistatin gene to muscles body-wide can preserve or restore strength and reduce disease symptoms in dystrophic mice. Secondly, I will study the changes in cell signaling that occur in the muscles of healthy and dystrophic mice following follistatin expression to determine if the signals that control muscle fiber growth are impaired in dystrophic muscles. Thirdly, I will investigate whether short-term administration of recombinant follistatin protein to dystrophic mice can enhance muscle strength without using viral vectors.

Parkville - Murdoch Children's Research Institute

Joseph Sarsero Ph.D.

(RG) Pharmacological therapies for Friedreich ataxia using cellular and mouse models

\$118,750 1/1/2010 12/31/2010 Year 3

Summary Our aim is to identify and develop new pharmacological approaches for the restoration of FRDA gene expression and the therapy of FRDA.

David Ross Thorburn Ph.D.

(RG) Mechanisms of cellular damage in mitochondrial oxidative phosphorylation disease

\$118,750 1/1/2010 12/31/2010 Year 2

\$118,750 1/1/2011 12/31/2011 Year 3

Summary Current treatments for inherited mitochondrial energy generation disorders are ineffective because we do not understand the disease mechanisms that cause damage to cells. Study of these is limited by the difficulty in accessing human tissues and the relative lack of suitable animal models. The overall aim of this application is to gain a greater understanding of the molecular mechanisms by studying cultured cells from patients with the most common energy generation disorder, complex I deficiency. We will also use other newly available model systems of complex I deficiency, including cultured brain cells from complex I deficient mice, and human adult stem cells and mouse embryonic stem cells with potential to be differentiated into cell types that reflect brain function more closely.

We will determine whether problems in calcium handling and oxidative stress offer new approaches to treatment of mitochondrial disease. Outcomes will be relevant not only for patients with such diseases, but also for our understanding and potential treatment of mitochondrial dysfunction in related conditions such as Friedreich ataxia.

Parkville - The University of Melbourne

Gordon Stuart Lynch Ph.D.

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

\$125,000	7/1/2010	6/30/2011	Year 1
\$125,000	7/1/2011	6/30/2012	Year 2
\$125,000	7/1/2012	6/30/2013	Year 3

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

Sydney - The University of Sydney

Des Richardson Ph.D., D.Sc.

(RG) The role of iron in Friedreich's ataxia and the use of iron chelation therapy

\$128,818	1/1/2010	12/31/2010	Year 3
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Summary My group has developed novel iron binding drugs that can remove toxic iron in FA mouse model (MCK) preventing heart problems seen in Friedreich's ataxia patients. We now want to assess if these new iron binding drugs can prevent the neural damage that is observed in another mouse model of Friedreich's ataxia which is known as NSE. Our aim in these studies is to develop a new drug for treatment of FA. This exciting work is currently supported by MDA and we hope that we can continue our studies in the present proposal.

BELGIUM

Gent - Flanders Institute for Biotechnology and University of Antwerp

Albena Jordanova Ph.D.

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

\$94,210	7/1/2010	6/30/2011	Year 1
\$94,210	7/1/2011	6/30/2012	Year 2
\$94,210	7/1/2012	6/30/2013	Year 3

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

CANADA**Manitoba****Winnipeg - University of Manitoba****Jiming Kong Ph.D.**

(RG) Rescue of motor neuron death in ALS by targeting the BNIP3 death gene family

\$102,298	1/1/2010	12/31/2010	Year 3
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Summary The present proposal is built on our previous studies to further define the BNIP3-induced cell death pathway and to test new neuroprotective strategies by targeting members of the BNIP3 death gene family. With greater understanding of mechanisms causing motor neuron cell death new and effective therapeutic strategies to protect against neuronal cell death in ALS may be identified.

Ontario**Ottawa - Ottawa Hospital Research Institute****Rashmi Kothary Ph.D.**

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

\$116,160	7/1/2009	6/30/2010	Year 1
\$116,160	7/1/2010	6/30/2011	Year 2
\$116,160	7/1/2011	6/30/2012	Year 3

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

Lynn Megeney Ph.D.

(RG) The role of caspase 3 in satellite cell self renewal

\$124,260	1/1/2010	12/31/2010	Year 1
\$124,260	1/1/2011	12/31/2011	Year 2
\$124,260	1/1/2012	12/31/2012	Year 3

Summary Growth and repair in post natal skeletal muscle is controlled by a stem cell population referred to as satellite cells. As such, satellite cells are the ideal source for repairing or replacing damaged skeletal muscle that is associated with injury or disease. Although the mechanisms that regulate satellite cell maturation into muscle fibers are well understood, we have little understanding of what controls the initial activation or renewal of these stem cells. Here we are investigating the role of the caspase 3 protein in satellite cell behavior. We have shown that caspase 3 limits the ability of satellite cells to remain as stem cells and encourages the maturation process to muscle. We propose to investigate the mechanisms by which caspase 3 impairs muscle stem cell self renewal.

Michael A. Rudnicki Ph.D.

(RG) Molecular regulation of satellite cell function

\$95,000	7/1/2009	6/30/2010	Year 2
\$95,000	7/1/2010	6/30/2011	Year 3

Summary Wnts are members of a large family of secreted proteins involved in regulating the formation and repair of tissues. We have found that overexpression of Wnt7a during skeletal muscle regeneration results in the marked enhancement of repair by stimulating the growth of muscle stem cells. In addition, we have noted that in the muscle of a mouse model of Duchenne Muscular Dystrophy, the muscle stem cell pool is apparently depleted. However, overexpression of Wnt7a is nevertheless capable of stimulating muscle repair in mdx muscle, suggesting that Wnt7a has additional activities. These experiments will determine the activities of the different Wnts expressed during regeneration, and establish at which level they function. This work will generate important new information concerning the underlying basis for the dysfunction of dystrophin-deficient muscle stem cells and explore the utility of Wnt delivery into dystrophic muscle as a potential strategy for the treatment of DMD.

Luc A Sabourin Ph.D.

(RG) Role of the Ste20 kinase SLK in myoblast migration and differentiation

\$109,250	7/1/2009	6/30/2010	Year 2
\$104,500	7/1/2010	6/30/2011	Year 3

Summary Migration of myogenic cells occurs extensively during skeletal muscle regeneration and is important in myoblast transfer therapy. Up to now, the treatment of muscular dystrophy using myoblast transfer into muscle tissue has had limited success due in part to the low dispersion of grafted cells outside the primary site of injection. Our laboratory has recently isolated a novel protein kinase, termed SLK, involved in the control of cell death and cellular reorganization. Our experiments will focus on understanding the molecular mechanisms that regulate cellular rearrangements and myoblast migration using in vitro and in vivo approaches. Dissection of the regulatory mechanisms that govern cellular reorganization and cell migration will contribute significantly to the design of more efficient repair therapies.

Ottawa - The University of Ottawa

Alexandre Blais Ph.D.

(RG) Characterization of the role of the Six1 transcription factor during myogenesis.

\$109,849 1/1/2010 12/31/2010 Year 3

Summary Our lab is interested in defining the transcriptional regulatory events that oversee skeletal muscle development. Elucidation of the network of molecular interactions that occur during myogenesis will reveal the identity of the key players and uncover their function in this complex process. This will allow us to reach our ultimate goal, which is to genetically reprogram cells, enhance and exploit their myogenic potential, and use them to restore muscle function in cell-based therapies of muscular illnesses.

Bernard Jasmin Ph.D.

(RG) Impact of exercise mimetics on the dystrophic pathology in the mdx mouse

\$120,000 7/1/2010 6/30/2011 Year 1

\$120,000 7/1/2011 6/30/2012 Year 2

\$120,000 7/1/2012 6/30/2013 Year 3

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

Aymeric Ravel-Chapuis Ph.D.

(DG) Role of the RNA-binding protein Staufen1 in myotonic dystrophy type 1

\$59,950 7/1/2010 6/30/2011 Year 1

\$59,950 7/1/2011 6/30/2012 Year 2

\$59,950 7/1/2012 6/30/2013 Year 3

Summary Myotonic Dystrophy type 1 (DM1) affects 1/8000 individuals worldwide and up to 1/500 in certain regions. The disease affects skeletal muscles, which become weak, painful and do not properly relax following contraction. It also affects other organs such as heart, eyes, nervous system, and endocrinal system. It is a genetic disorder caused by a mutation, a repetition of CTG trinucleotides, in the DMPK gene. The pathological RNA expressed from this gene is blocked into the nucleus of the cell where it aggregates. It becomes toxic to the cell because it sequesters proteins, preventing them from assuming their normal functions and thereby causing the many symptoms characteristic of this disease. The current proposal is designed to examine the role of one such protein, called Staufen. Our work shows that Staufen interacts with the DMPK RNA, and that the modulation of Staufen levels in DM1 cells can affect mutant RNA accumulation in the nucleus and revert some features observed in the disease. The identification of such a protein and the elucidation of its functions in skeletal muscle is important since these

studies may lead to the development of new therapeutic strategies for treating DM1.

Ilona Skerjanc Ph.D.

(RG) Enhanced muscle repair with human embryonic stem cells

\$114,000	1/1/2010	12/1/2010	Year 2
\$114,000	1/1/2011	12/1/2011	Year 3

Summary Cell therapies to reverse muscle atrophy and to strengthen skeletal muscle would greatly enhance and extend the lives of patients with dystrophic diseases, including muscular dystrophy. Several cell sources for therapy are currently under study by others, including satellite, adult stem, and mesenchymal stem cells. However, difficulties have been encountered with these approaches, including the requirement for invasive procedures, the availability of suitable donors, and the limited long-term proliferation potential. In contrast, human embryonic stem (hES) cells could provide an unlimited number of cells, with an enhanced proliferation ability. A recent study has reported the isolation of skeletal myoblasts from differentiating hES cells and their transplantation into mice, indicating the potential of this approach for future therapeutic applications. However, the ability of hES-derived myoblasts to contribute to the satellite cell niche and to enhance skeletal muscle function was not assessed. To this end, we will use a novel method to isolate a skeletal myoblast/progenitor population from hES cells and examine their ability to engraft into skeletal muscle in mdx mice, assessed by their contribution to the satellite cell niche, and enhancement of muscle function. The overall goal is to provide a method of hES cell differentiation and enrichment that will generate human myoblasts/progenitors for long-term engraftment and future therapeutic applications.

Québec

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

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

(RG) Whole exome sequencing in patients with familial ALS

\$135,653	1/1/2010	12/31/2010	Year 1
\$135,653	1/1/2011	12/31/2011	Year 2
\$135,653	1/1/2012	12/31/2012	Year 3

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

Montreal - CRCHUM - Hopital Notre-Dame

Bernard Brais M.D.

(RG) Studying the role of mutated integrins in two new forms of muscular dystrophies

\$90,000 1/1/2010 12/31/2010 Year 2

Summary We have recruited two distinct cohorts of French-Canadian families affected by two new forms of MD. A first grant from the MDA allowed the original identification of mutations in an integrin in a cohort of 16 individuals from 13 families affected by a novel congenital muscular dystrophy with hyperlaxity (CMDH). In parallel, we have identified a probable mutation in another integrin in families of Acadian ancestry affected by a new childhood onset limb girdle muscular dystrophy (LGMD). These MD are only the second and third to be found to be caused by mutations in an integrin since the discovery in 1998 of mutation in ITGA7 in a very rare form of congenital MD. Integrins form a large family of transmembrane proteins involved in numerous functions. Their function in muscle is still not fully understood. We will investigate how mutations in these two integrins cause these new forms of MD and explore in more general the roles of integrins in normal muscle development and function.

Bernard Brais M.D.

(RG) Characterization of the function of TMEM16E/ANO5 gene mutated in LGMD2L

\$84,000 7/1/2009 6/30/2010 Year 1

\$84,000 7/1/2010 6/30/2011 Year 2

Summary To date 17 genes have been identified to be mutated in different forms of limb-girdle muscular dystrophy (LGMD). We previously described a cohort of eight French-Canadian patients displaying the new form of limb-girdle muscular dystrophy 2L (LGMD2L, MIM 611307), characterized by prominent quadriceps atrophy and myalgia. A previous MDA grant allowed the mapping of this new form to chromosome 11 and the uncovering of mutations in the transmembrane protein 16E/anoctamin5 (TMEM16E/ANO5) gene. TMEM16E's function is unknown but it is highly expressed in skeletal muscle and more highly expressed in mdx mice muscle. Recently, TMEM16A/ANO1 was found to act as a calcium-activated chloride channel, suggesting that TMEM16E may have a similar function in skeletal muscle. The research program is organized around three specific aims: 1) complete the clinical, pathological and genetic characterization of LGMD2L; 2) characterize the putative ion channel function of TMEM16E/ANO5 and how it is modified in LGMD2L; and 3) uncover protein partners of TMEM16E/ANO5 in muscle. Uncovering the role of TMEM16E in skeletal muscle will shed new light onto an uncharted field of muscular dystrophy pathophysiology in which ion channels may play a role in preserving calcium-regulated membrane repair.

Edor Kabashi Ph.D.

(DG) Functional characterization of TDP-43 mutations in amyotrophic lateral sclerosis

\$60,000 7/1/2009 6/30/2010 Year 1

\$60,000 7/1/2010 6/30/2011 Year 2

\$60,000 7/1/2011 6/30/2012 Year 3

Summary The genetic cause of only 1-2% of all ALS cases is accounted for by mutations in the SOD1 gene. Consequently the identification of novel ALS-causative genes remains imperative. TDP-43 has been demonstrated to be a major component of the protein aggregates seen in ALS patients but no mutation in the TARDBP gene that encodes TDP-43 protein had been identified. Our group and

several other research teams have recently identified and genetically validated mutations of TARDBP in sporadic and familial ALS cases. This suggests that mutant TDP-43 is the sole cause of ALS in a subset of patients. This project will help to determine and understand the negative impact of TARDBP mutations in ALS and motor neuron degeneration. In order to understand the toxic aspects of mutant TDP-43 I am currently establishing appropriate models in cells (motor neurons from spinal cord cultures) and living organisms (zebrafish). Functional characterization of mutant TDP-43 in these models will help to determine what the molecular partners of mutant TDP-43 are as well as the role this protein normally plays in motor neuron biology. Finally, these novel models will be used to screen a large number of pharmaceutical compounds that should directly open avenues for the development of therapies that could delay or prevent disease onset and progression in all ALS patients.

Montreal - McGill University

Heather D. Durham Ph.D.

(RG) Common pathogenic factors in motor neuron diseases as targets for intervention

\$102,295	7/1/2009	6/30/2010	Year 2
\$103,753	7/1/2010	6/30/2011	Year 3

Summary Mutations that cause certain inherited forms of ALS and other neurological diseases that affect motor neurons, such as Kennedy's disease and Charcot-Marie-Tooth disease (CMT) have been identified. By expressing those mutant genes in cultured cells and animals, scientists have created experiment models to explore how the mutant proteins cause dysfunction and even death of motor neurons. To improve our ability to identify, test and predict the usefulness of a potential therapy, we can identify the critical ways that these various mutant proteins disrupt the function of neurons, and focus on those that occur early and in multiple diseases. We can determine how candidate drugs prevent these early difficulties as well as more disastrous consequences, including motor neuron death and loss of locomotion. We can improve our screening methods to more quickly identify the best therapies to move forward for further testing. This research project will address those goals by improving upon our primary culture models of ALS, CMT and Kennedy's disease to measure and understand early, subtle changes in mitochondria (energy producing factories in cells) movement of intracellular traffic, and control of intracellular calcium, and by determining how therapeutic candidates intervene with these more subtle aspects of motor neuron function.

Josephine Nalbantoglu Ph.D.

(RG) Artificial zinc fingers targeting the human utrophin promoter

\$104,390	7/1/2010	6/30/2011	Year 1
\$104,390	7/1/2011	6/30/2012	Year 2
\$104,390	7/1/2012	6/30/2013	Year 3

Summary In Duchenne Muscular Dystrophy (DMD), repeated cycles of muscle fiber destruction lead to progressive paralysis and death. The basic cause of this is the lack of an essential protein, dystrophin, at the surface membrane of muscle fibers due to a mutation of a very large gene on the X chromosome. A promising approach to the therapy of DMD is the transfer into muscle fibers of a normal dystrophin gene by viral and non-viral vectors. Another molecular approach to DMD therapy is a substantial increase of an analogue of dystrophin, utrophin, so that it is

expressed not only at its normal site of the neuromuscular junction but throughout the sarcolemma. Several pieces of evidence in mouse and dog models of DMD indicate that a substantial increase of the amount of the extrasynaptic utrophin will mitigate or eliminate muscle fiber damage caused by dystrophin deficiency. We have previously published that artificial transcription factors that target the promoter of mouse utrophin can also result in increased levels of utrophin promoter and mitigate muscle fiber damage. In this proposal, we will use the same approach to design artificial transcription factors which target the human utrophin promoter. It is hoped that these may eventually be used as part of therapy for Duchenne muscular dystrophy.

Montreal - Montreal Neurological Institute

Ken Hastings Ph.D.

(SG) The George Karpati Symposium on Neuromuscular Disease (May 10, 2010)
 \$2,000 5/1/2010 4/30/2011 Year 1

Summary The George Karpati Symposium on Neuromuscular Disease: Innovation and Application will be held on May 10, 2010 at the Montreal Neurological Institute, McGill University. This one-day scientific symposium has been organized as a tribute to the life's work of George Karpati, the noted neurologist and clinician-scientist who died suddenly and unexpectedly in Montreal in February 2009, abruptly ending a distinguished career in neuromuscular disease research at the Montreal Neurological Institute. The symposium will bring together international leaders in neuromuscular disease research and clinical application for a full day's program of scientific presentations. Speakers are Drs Stanley Appel, Valerie Askanas, Andrew G. Engel, W.K. Engel, Kevin Flanigan, R.C. Griggs, Hanns Lochmuller, Guy Rouleau, and Eric Shoubridge, with additional presentations by Zohar Argov and Bernard Brais. The target audience is practicing clinicians, researchers, and trainees. We expect an attendance in the range of 150-200. We have arranged for CME credit for the Karpati Symposium through McGill University. Our overall cost estimate for the symposium is ~\$29,000, of which we request herein \$2,000 as an MDA Special Grant. In terms of significance to MDA, we believe the Karpati symposium will be an important forum for disseminating expert information on the current state of neuromuscular disease research and treatment.

Montréal - Institut de Recherches Cliniques de Montréal

Artur Kania Ph. D

(RG) Motor neuron survival factors in development
 \$113,121 7/1/2009 6/30/2010 Year 2
 \$113,121 7/1/2010 6/30/2011 Year 3

Summary Many motor neuron disorders are characterized by motor neuron loss leading to paralysis and eventual patient death. Thus, blocking motor neuron death through administration of neuronal survival factors is a key strategy for therapeutic approaches. Many neuronal survival factors have been identified by studying normal neuronal death that occurs in the developing nervous system, but how such factors work to prevent neuronal death is still unclear. My laboratory is studying the development of spinal cord motor neurons that innervate and control limb muscles. The objective of this study is a detailed characterization of signals secreted by muscles that prevent the death of motor neurons during embryonic development. We anticipate that this research will (1) tell us whether different classes of motor

neurons require specific combinations of survival factors to stay alive, and (2) possibly lead to new therapies aimed at improving motor neuron survival in neurodegenerative diseases such as Spinal Muscular Atrophy or Amyotrophic Lateral Sclerosis.

Quebec - Laval University

Francois Berthod Ph.D.

(RG) Development of a tissue-engineered model of spinal cord to study amyotrophic lateral sclerosis

\$71,673 7/1/2009 6/30/2010 Year 3

Summary Researchers will construct a 3-D cellular model to test various factors influencing survival of these cells in FALS.

Jean-Pierre Julien Ph.D.

(RG) Chromogranin variants as risk factor and modifier on disease onset for ALS

\$115,000 7/1/2010 6/30/2011 Year 1

\$115,000 7/1/2011 6/30/2012 Year 2

\$115,000 7/1/2012 6/30/2013 Year 3

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

CHILE

Santiago - Pontificia Universidad Catolica de Chile

Enrique Brandan Ph.D.

(RG) CTGF the factor involved in fibrosis development in DMD

\$64,671 7/1/2009 6/30/2010 Year 2

\$66,819 7/1/2010 6/30/2011 Year 3

Summary Reports suggest an increase in connective tissue growth factor (CTGF) mRNA in dystrophic dogs, and microarray analysis, indicate an enhanced level of CTGF in mdx mice. Although the participation of CTGF in fibrosis has gained attention in the last years, little information regarding the function of CTGF in skeletal muscle fibrosis is available. The main goal of this project is to elucidate the biological role of CTGF, decorin and LRP on skeletal muscle fibrosis. Experiments are designed to: genetically determine whether CTGF is responsible for fibrosis development in dystrophic muscles; determine the possible complex formation between LRP-decorin-CTGF and its role in muscle fibrosis; evaluate the inhibitory interaction between some proteoglycans and CTGF; identify activators and/or inhibitors of CTGF obtained using CTGF affinity chromatography followed by proteomic analyses; study the CTGF gene promoter region and several putative

response elements, particularly those related with skeletal muscle formation, atrophy and inflammation. This project will provide important basic information on the mechanisms underlying fibrosis development in skeletal muscle and will allow us to identify possible target molecules that interfere with fibrosis.

GERMANY

Hannover - Medizinische Hochschule Hannover (MHH)

Christoph M. Fahlke M.D.

(RG) Chloride channel dysfunction in myotonia congenita

\$115,234 1/1/2010 12/31/2010 Year 2

\$119,842 1/1/2011 12/31/2011 Year 3

Summary Myotonia congenita is a genetic human muscle disease characterized by muscle stiffness upon sudden forceful movement. The life-long muscle stiffness can be quite disabling, and the current treatment is largely ineffective and poorly tolerated by many patients. The molecular cause of this disease has been well understood for many years. Both autosomal dominant and recessive myotonia congenita are caused by mutations in the gene encoding the principal human skeletal muscle Cl⁻ channel, hCIC-1 (CLCN1 gene). However, information linking genetic information to clinical symptoms is still missing. We will study the effects of a large number of CLCN1 mutations found in myotonia congenita on various aspects of muscle chloride channel function. We will evaluate how mutant channels conduct anion and open and close upon changes of the membrane potential. Disease-causing mutations might also affect the number of channels in the muscle fiber or its subcellular distribution. Lastly, mutations might impair the ability of CIC-1 proteins to form dimeric chloride channels. We will study these different functions and correlate them to clinical symptoms of the respective patients. We expect from these studies insights into the molecular determinants of the various symptoms of myotonia congenita. This information will be important for identifying novel pharmacological treatments for myotonia congenita.

GREECE

Athens - Hellenic Pasteur Institute

Socrates J. Tzartos Ph.D.

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

\$116,700 1/1/2010 12/31/2010 Year 1

\$116,700 1/1/2011 12/31/2011 Year 2

\$116,700 1/1/2012 12/31/2012 Year 3

Summary Myasthenia gravis (MG) is a neuromuscular disease caused mainly by an autoimmune response against the muscle nicotinic acetylcholine receptor (AChR) which interferes with neuromuscular transmission. In MG patients, autoantibodies lead to loss of AChRs, resulting in impaired neuromuscular transmission reflected by weakness and fatigability of voluntary muscles. Current treatments are non-specific and most are associated with severe side-effects. The extensive and valuable information available on MG makes this disease a most appropriate target for the development of an antigen-specific therapy. Our main aim is to develop an MG-specific therapeutic approach aimed at the selective depletion of the Abs from patients' blood and the return of the cleared blood to the patient. This project is

based on our very encouraging results for the clearance of MG sera using immobilized recombinant extracellular domains (ECDs) of the five AChR subunits, and has three specific aims: I) To increase the immunoadsorption efficiency of the ECDs by the expression of native-like pentamers assembled from the five ECDs. II) To investigate the in vivo effectiveness of Ab depletion by studying the pathogenicity of the untreated sera, Ab-depleted sera, and the purified Abs in rodents. III) To scale up the immunoadsorption procedure and initiate preliminary clinical trials.

ISRAEL

Jerusalem - Hebrew University of Jerusalem

Yosef Gruenbaum Ph.D.

(RG) Mechanism of emerin and LEM-2 regulation and function

\$60,087 7/1/2009 6/30/2010 Year 3

Summary Investigators will study overlap of emerin and other LEM-domain proteins to determine factors in X-linked EDMD

Orna Halevy Ph.D.

(RG) Inhibition of fibrosis and mode of action of halofuginone in muscle dystrophies

\$111,815 1/1/2010 12/31/2010 Year 3

Summary We discovered the clinical potential of halofuginone (halo) as a novel anti-fibrotic therapy. By inhibiting fibrosis halo protects against muscle damage and lessens the need for excessive muscle regeneration. In this proposal we will evaluate the effect of halo on muscle fibrosis in a CMD mouse model (dy2J/dy2J). Success in the study would demonstrate that halo treatment would be beneficial for other MDs

Raanana - Open University of Israel

Miriam Souroujon Ph.D.

(RG) Suppression of myasthenia gravis by regulatory T cells: studies in EAMG

\$104,254 1/1/2010 12/31/2010 Year 2

\$103,198 1/1/2011 12/31/2011 Year 3

Summary We aim to develop improved immunotherapies for the autoimmune neuromuscular disease myasthenia gravis (MG) that would not have the adverse side effects of the treatments used today. Our studies are conducted mainly in experimental autoimmune MG (EAMG) in rats as a model. The proposed project focuses on regulatory T cells (Treg) that are known as key players in maintaining immune tolerance and were reported to display impaired function in MG, suggesting their involvement in the immunopathology of the disease and their potential in its therapy. Our recent data point to differences between Treg from EAMG rats and healthy controls and demonstrate that Treg from healthy donors can suppress EAMG. This proposal is aimed at extending these studies along the following lines: further analyze qualitative and quantitative differences between Treg in EAMG and healthy controls, assess the contribution of the equilibrium between the suppressive Treg and the pathogenic Th17 cells to the susceptibility and course of myasthenia and understand the importance of antigen specificity for the suppressive activity of Treg. These analyses will lead to in vivo studies in which we will use Treg from sick

syngeneic or healthy allogeneic donors to treat EAMG. Finally, the humanized SCID/NOD mouse model will be used to test the therapeutic potential of autologous and allogeneic Treg in a system closer to human MG. Hopefully, these studies will lead to Treg-based clinical trials in MG.

Rehovot - Weizmann Institute of Science

Ben-Zion Shilo Ph.D.

(RG) WASp-based actin polymerization promotes myoblast fusion in drosophila and mice

\$100,000	7/1/2009	6/30/2010	Year 1
\$100,000	7/1/2010	6/30/2011	Year 2
\$100,000	7/1/2011	6/30/2012	Year 3

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

Tel Aviv - Tel-Aviv University

Daniel Offen Ph.D.

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

\$119,900	7/1/2010	6/30/2011	Year 1
\$119,900	7/1/2011	6/30/2012	Year 2
\$119,900	7/1/2012	6/30/2013	Year 3

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

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

ITALY

Genova - Fondazione Istituto Italiano di Tecnologia

Maria Pennuto Ph.D.

(DG) Targeting androgen receptor for development of SBMA therapeutics

\$59,691 7/1/2009 6/30/2010 Year 2

\$59,976 7/1/2010 6/30/2011 Year 3

Summary Spinobulbar muscular atrophy (SBMA) is caused by expansion of a CAG sequence, encoding glutamine, to over 38 residues in the androgen receptor (AR) gene. In SBMA only males are fully symptomatic because mutant AR becomes toxic only in the presence of ligand, testosterone. However, little is known about how the ligand converts the protein into a toxic species. Based on preliminary findings, we hypothesize that phosphorylation, one of the ligand-induced modifications, alters toxicity of mutant androgen receptor. We have previously shown that phosphorylation by Akt reduces toxicity of mutant AR. We have now obtained evidence that another kinase, PKA, reduces mutant AR toxicity in the cell. We will survey the effect of activation of PKA signaling pathway on SBMA cells. We have preliminary evidence that IGF-1, which initiates the Akt signaling, ameliorates the phenotype of SBMA mice. We intend to confirm the effect of IGF-1 in mice with histopathological and behavioral analyses, and to develop methods to safely and efficiently deliver IGF-1 as therapy for SBMA.

Milano - Fondazione Centro San Raffaele del Monte Tabor

Davide Gabellini Ph.D.

(RG) Characterization of the molecular mechanisms altered in FSHD

\$105,545 1/1/2010 12/31/2010 Year 2

\$98,230 1/1/2011 12/31/2011 Year 3

Summary Facioscapulohumeral muscular dystrophy (FSHD) is associated with reduction in the number of copies of DNA on chromosome 4, called D4Z4, that is repeated many times toward the end of the long arm of chromosome 4. We hypothesized that D4Z4 may control the activity of nearby FSHD genes. We found that in FSHD patients there is an increased production of the proteins encoded by the genes close to D4Z4. Interestingly, we found that these proteins are over-produced specifically in the muscles of FSHD patients explaining the fact that FSHD is primarily a disease of skeletal muscle. More recently, with the idea of modeling in an animal the same conditions observed in FSHD patients, we generated mice over-producing the same proteins that are over-produced in the muscles of FSHD patients. We found that mice over-producing a protein called FRG1 display several features of FSHD patients. Based on these results, we propose that loss of D4Z4 causes over-production of FRG1, which leads to FSHD. We aim to: - understand the molecular mechanism responsible for increased production of 4q35 proteins in FSHD - understand the specific processes that go awry in muscles of patients suffering from FSHD.

Roma - Provincia Italiana CFIC-Istituto Dermopatico dell' Immacolata

Carlo Gaetano M.D.

(RG) HDAC inhibitors as experimental therapeutics in Duchenne cardiomyopathy
\$26,600 7/1/2009 6/30/2010 Year 2
\$25,650 7/1/2010 6/30/2011 Year 3

Summary The lack of functional dystrophin predisposes DMD patients to contraction-induced muscle cell disruption. In skeletal muscle, satellite cells participate in muscle regeneration, producing newly formed myotubes. This process counterbalances degeneration in the first phase of the disease. However, the excessive myogenic cell division results in accelerated senescence, which is a leading cause of the progressive skeletal muscle failure. Currently it is unknown whether similar pathogenetic mechanisms play a role in DMD cardiomyopathy (CM). Recently we reported that histone deacetylase inhibitors (HDACi) are beneficial, improving skeletal muscle regeneration in MDX mice, acting at least in part on satellite cell proliferation and differentiation. Little is known, however, about their effect on cardiac muscle. Our preliminary evidence indicates that prolonged treatment with suberoylanilide hydroxamic acid (SAHA), a pan-HDACi suitable for human use, reduced the number of ventricular arrhythmias in MDX mice exposed to different social challenges. The aim of this project is to establish in vivo and at cellular and molecular levels whether and how HDACi treatment may improve the cardiac muscle performance in the MDX mouse model of Duchenne muscular dystrophy.

MACEDONIA

Veles - Faculty of Technology and Technical Sciences

Darko Bosnakovski D.V.M., Ph.D.

(DG) Molecular analysis of DUX4 and gene therapy for FSHD
\$45,000 7/1/2009 6/30/2010 Year 3

Summary In FSHD, DUX4 gene expression is expressed only in myoblasts of affected patients. Studies will attempt to understand DUX4 effects on downstream target genes.

NETHERLANDS

Leiden - Leiden University Medical Center

Silvere Maria van der Maarel Ph.D.

(RG) The developmental role of D4Z4 in FSHD pathogenesis
\$81,169 7/1/2009 6/30/2010 Year 3

Summary Using transgenic mice, studies will address issues of chromatin structure and DNA stability in FSHD.

Silvere Maria van der Maarel Ph.D.

(RG) AONs to modulate the transcriptional landscape of D4Z4
\$99,323 4/1/2010 3/31/2011 Year 1
\$99,746 4/1/2011 3/31/2012 Year 2

Summary Facioscapulohumeral muscular dystrophy (FSHD) is caused by contractions of the D4Z4 repeat on the 4A161 permissive haplotype of the

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

Silvere Maria van der Maarel Ph.D.

(SG) Best Practice Meeting for the DNA Diagnosis of FSHD

\$6,250	6/1/2010	6/30/2010	Year 1
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Summary Over the past years we have experienced an ever increasing complexity in the DNA diagnosis of FSHD. While 95% of patients can be diagnosed by Southern blot analysis of the D4Z4 repeat on chromosome 4q, this analysis is not uniformly performed and often gives reason for different interpretations. With the recent efforts in the muscular dystrophy field, including FSHD, to harmonize patient data collection internationally in large registries, it is important to also harmonize DNA diagnosis of FSHD. The last best practice meeting on the diagnosis of FSHD was organized in 1999 in Leiden. With the more recent advances in our understanding of the genetic defect in FSHD, a best practice meeting is long due. On June 9th of this year will organize a one-day best practice meeting in Leiden for which we have invited diagnostic laboratories worldwide to participate, share their experiences and get updated with the latest insight. We aim to generate a consensus on the genetic testing for FSHD which we aim to disseminate in a manuscript. We received a good response with already approximately 30 confirmed participants from The Netherlands, US, UK, Canada, Japan, France, Germany, Spain, Israel, Italy, Belgium, and Argentina. Confirmed speakers include Dr. Richard Lemmers, (Leiden, Netherlands), Dr. Bert Bakker (Leiden, Netherlands), Dr. Peter Lunt (Bristol, UK), Dr. Nicolas Lévy (Marseille, France), Dr. Rossella Tupler (Modena, Italy), and Yukiko Hayashi (Japan).

Nijmegen - Radboud University Nijmegen Medical Centre

Berend Wieringa Ph.D.

(RG) Oligonucleotide-mediated silencing of (CUG)_n RNA stress in myotonic dystrophy

\$134,261	7/1/2009	6/30/2010	Year 1
\$131,661	7/1/2010	6/30/2011	Year 2
\$130,061	7/1/2011	6/30/2012	Year 3

Summary The main burden of myotonic dystrophy type 1 (DM1) is the continuous worsening of muscle, brain and cardiac problems. This progressive nature can be explained by (i) ongoing somatic expansion of the (CTG•CAG)_n repeat in the DMPK gene and (ii) cellular toxicity of abnormal gene transcripts causing misregulation of normal cellular programming. One may expect that treatment aimed at reducing these RNA-based effects will alleviate the main problems of DM1. In this proposal, a recently developed strategy based on selective

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

SINGAPORE

Singapore - National University of Singapore

Reshma Taneja Ph.D.

(RG) Regulation of skeletal muscle regeneration by Stra13

\$102,640 7/1/2009 6/30/2010 Year 2

\$105,305 7/1/2010 6/30/2011 Year 3

Summary Myofibers in DMD patients undergo continuous cycles of degeneration and regeneration leading to necrosis, fibrosis and inflammation. The identification of genes involved in muscle regeneration is critical to design therapeutic approaches for muscular dystrophies. We have identified deregulated signaling of two critical pathways in Stra13 mutant mice that may contribute to defective regeneration of muscle. We will examine the relative contribution of each pathway in defective regenerative capacity of Stra13^{-/-} mice. We expect that these studies will lead to novel therapeutic targets and impact on our understanding of the molecular basis of muscular dystrophies.

SPAIN

Barcelona - Universitat Pompeu Fabra

Pura Munoz Canoves Ph.D.

(RG) Therapeutic fibrinolysis for Duchenne muscular dystrophy in mdx mice

\$95,000 7/1/2009 12/31/2010 Year 3

Summary Investigators will test anrod therapy to reduce fibrin in DMD models to determine if progression may be slowed.

SWEDEN

Lund - Experimental Medical Science, Lund University

Madeleine Durbeej-Hjalt Ph.D.

(RG) Laminins and congenital muscular dystrophy

\$130,571 1/1/2010 12/31/2010 Year 3

Summary The aim of this project is to develop therapies for MDC1A lacking laminin alpha2. We will test whether similar laminins can function equally well as laminin alpha2 chain in muscle to avoid immune reactions. We will study a potential

signaling molecule. These studies will provide new insights into laminin alpha2 chain signaling and open potential avenues for therapy of MDC1A.

SWITZERLAND

Basel - University of Basel

Christoph Handschin Ph.D.

(RG) Amelioration of muscle atrophy and Duchenne muscular dystrophy by PGC-1alpha

\$105,355 1/1/2010 12/31/2010 Year 3

Summary The peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) has been shown to be a key regulator of the adaptations of skeletal muscle to endurance exercise. We observed that mice with elevated PGC-1alpha are resistant to disuse-induced muscle atrophy and have markedly improved muscle function when crossed to a mouse model of Duchenne muscular dystrophy. We therefore aim at expanding our knowledge about PGC-1alpha in skeletal muscle in order to identify more attractive drug targets that are linked with the PGC-1alpha-mediated improvement of muscle atrophy and dystrophies.

UNITED KINGDOM

London - Institute of Child Health

Francesco Muntoni M.D.

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

\$125,000 7/1/2010 6/30/2011 Year 1

\$125,000 7/1/2011 6/30/2012 Year 2

\$125,000 7/1/2012 6/30/2013 Year 3

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

London - Institute of Neurology, UCL

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

(DG) Genetic Modifiers of the CMT1A phenotype
\$60,000 1/1/2010 12/31/2010 Year 2
\$60,000 1/1/2011 12/31/2011 Year 3

Summary CMT1A is caused by having an extra copy of a region on chromosome 17 and patients have a wide range of severities and age of onset. This suggests there are other genetic and environmental factors that affect CMT1A. These factors are very important, as they will affect all types of CMT and neuropathy in general. The gene(s) that effect CMT1A will be important therapeutic targets. We plan to select 250 British, 350 American, 200 Italian and 200 Brazilian CMT1A patients with characterized clinical and electrical features. We will look to analyze patients over the full spectrum of disease severity and age of onset. In these cases we will carry out a whole genome polymorphism and expression study on DNA in each patient and on mRNA extracted from Sural nerve biopsies from patients to identify which genetic regions are associated with the more severe or milder types of CMT1A.

London - Royal Veterinary College**Susan Carol Brown Ph.D.**

(RG) FKRP knock-in mice as a model for the pathogenesis of the dystroglycanopathies
\$118,750 1/1/2010 12/31/2010 Year 3

Summary We have now generated mice which carry a mutation in FKRP. When this mutation is found in human patients it is associated with muscular dystrophy (dystroglycanopathy). This unique animal model will provide us with the means to understand the pathogenesis of the most common dystroglycanopathy and also allow us to assess therapeutic intervention to improve the clinical features which characterise FKRP mutant patients and mice.

Oswestry - Robert Jones & Agnes Hunt Hospital**Glenn Eric Morris D. Phil.**

(TRIG) The MDA Monoclonal Antibody Resource
\$117,176 10/1/2009 9/30/2010 Year 3

Summary Professor Morris's Biochemistry Group has, over the past 20 years, built up a library of monoclonal antibodies for neuromuscular disease research, diagnosis and clinical trials. A collection of over 150 exon-specific antibodies against dystrophin is, and will continue to be, especially useful internationally in trials of potential therapies for Duchenne muscular dystrophy. Very popular antibodies have also been produced for research, diagnosis and drug evaluation in spinal muscular atrophy, Emery-Dreifuss muscular dystrophy, laminopathies and myotonic dystrophy. This project will ensure that these antibodies will continue to be available to researchers for the foreseeable future and will also add to and refine the library.

Oxford - University of Oxford**Kay Elizabeth Davies MA, Ph.D.**

(RG) Approaches to therapy for DMD
\$165,814 1/1/2010 12/31/2010 Year 1
\$107,883 1/1/2011 12/31/2011 Year 2

\$107,883 1/1/2012 12/31/2012 Year 3

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

Staffordshire - Keele University

Glenn Eric Morris D. Phil.

(TRIG) The MDA Monoclonal Antibody Resource for Neuromuscular Disease

\$127,985	10/1/2010	9/30/2011	Year 1
\$122,744	10/1/2011	9/30/2012	Year 2
\$124,696	10/1/2012	9/30/2013	Year 3

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

UNITED STATES

ARIZONA

Phoenix - St. Joseph's Hospital & Medical Center

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

(RG) Th17 cells in myasthenia

\$150,000	1/1/2010	12/31/2010	Year 1
\$150,000	1/1/2011	12/31/2011	Year 2
\$150,000	1/1/2012	12/31/2012	Year 3

Summary B cells are a fundamental component of the immune system. Anti-acetylcholine receptor (AChR)-antibodies are a product of B cells that cause

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

Tempe - Arizona State University

N. Jeanne Wilson-Rawls Ph.D.

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

\$117,152	1/1/2010	12/31/2010	Year 2
\$122,033	1/1/2011	12/31/2011	Year 3

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

Tucson - University of Arizona

Ronald E. Allen Ph.D.

(RG) The role of CXCR4 and stromal-derived factor 1 in satellite cell migration

\$132,025	1/1/2010	12/31/2010	Year 1
\$133,761	1/1/2011	12/31/2011	Year 2

Summary Skeletal muscle satellite cells are the primary stem cell associated with muscle fiber repair following damage in diseased muscle. An important aspect of satellite cell function is the ability to be activated from a rather dormant state and migrate to the site of fiber injury. The nature of the signals that tell satellite cells to migrate and that direct satellite cells to the site of damage is not completely clear. We have preliminary evidence that the CXCR4 receptor, which mediates cell migration in other cells types, is found in satellite cells after they are activated. This

project is designed to explore the role of this receptor and the factor that activates it, stromal-derived factor 1 (SDF1), in directing satellite cells to damaged fibers so that they can participate in rebuilding the diseased fiber.

Daniela Zarnescu Ph.D.

(RG) Gene and drug discovery in a drosophila model of ALS			
\$125,000	7/1/2010	6/30/2011	Year 1
\$125,000	7/1/2011	6/30/2012	Year 2
\$125,000	7/1/2012	6/30/2013	Year 3

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

CALIFORNIA

Davis - University of California

Gino Cortopassi Ph.D.

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

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

Michael Ferns Ph.D.

(RG) Regulation of AChR turnover and implications for diseases of NMJ

\$125,565	7/1/2009	6/30/2010	Year 1
\$125,565	7/1/2010	6/30/2011	Year 2
\$125,565	7/1/2011	6/30/2012	Year 3

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

Craig McDonald M.D.

(CRNG) MDA Clinical Research Network at UC Davis

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary The MDA Neuromuscular Disease Clinic at UC Davis will be a regional clinical research center as part of a MDA Clinical Research Network that supports studies of Duchenne muscular dystrophy (DMD). The UC Davis center will work with the MDA Clinical Research Network to: 1) Assume a leadership role in the development of a DMD clinical registry that will facilitate the conduct of clinical studies aimed at optimizing and standardizing clinical care; 2) Develop and optimize standardized and comprehensive outcome measures for DMD to be used in clinical studies and trials; 3) Refine and validate an innovative self-report health-related quality of life measure (NeuroQoL) which will be employed across the lifespan of children and adults with DMD; 4) Assess the relationship of candidate clinical endpoints to health-related quality of life measures in DMD to facilitate and expedite the testing of new therapies through clinical trials; 5) Coordinate activities which enhance communication and collaboration with MDA clinics in the western United States by engaging MDA clinics in training activities, regional education conferences, and collaborative clinical research activities.

David Paul Richman M.D.

(RG) Pathogenesis of anti-MuSK myasthenia

\$118,750	1/1/2010	12/31/2010	Year 2
\$118,750	1/1/2011	12/31/2011	Year 3

Summary The majority of, but not all, patients with myasthenia gravis (MG) have antibodies (Abs) that attack the acetylcholine receptors (AChR) at their nerve-

muscle junctions and block the signaling between the nerves and muscles. Other MG patients, many of whom have significant muscle wasting, have a different set of autoantibodies targeting another protein component of the nerve-muscle junction: muscle-specific kinase (MuSK). The role of Abs to MuSK in this form of MG is not known, in contrast to the role of Abs to the AChR in standard MG which are known to block the signaling between nerve and muscle. It is not even clear if the MuSK autoantibodies play any role in the myasthenia that develops in these patients. This project is aimed at determining whether these Abs are important and, if they are, precisely what effect they have on nerve-muscle signaling. We have successfully engineered tissue culture cells to produce MuSK and have purified the large amounts of the protein required for immunization of experimental animals to produce an animal model of this disease. We have recently immunized laboratory rats with the purified MuSK and have succeeded in producing a very severe form of this disease. We are now proposing to study the mechanisms by which the Abs cause this severe muscle weakness and wasting. This information will have a high likelihood of providing the types of information needed to develop treatments of this very severe and poorly understood form of MG.

Irvine - University of California

Virginia Kimonis M.D., MRCP

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

\$124,000	7/1/2010	6/30/2011	Year 1
\$124,000	7/1/2011	6/30/2012	Year 2
\$124,000	7/1/2012	6/30/2013	Year 3

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

Jouni Vesa Ph.D.

(DG) Characterization of molecular pathogenesis of IBMPFD

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary IBMPFD (Inclusion Body Myopathy associated with Paget's disease of bone and Frontotemporal Dementia) is caused by mutations in the VCP (Valosin Containing Protein) gene. The majority of disease mutations change a conserved amino acid 155 (R155H/P/C/S) suggesting an important role for this residue. The entire protein is also highly conserved, suggesting that VCP is necessary for normal development and survival of muscle, bone and brain cells. VCP is involved in several cellular activities including membrane fusion, transcription activation, cell

cycle control, apoptosis, and protein degradation. The aim of this project is to characterize those pathological cascades that result in muscle weakness in IBMPFD patients, using molecular and cellular approaches. We aim to clarify the pathological mechanisms of IBMPFD using cells and tissues from patients and control subjects, as well as from our recently generated mouse model for IBMPFD.

La Jolla - Ludwig Institute for Cancer Research

Don Cleveland Ph.D.

(RG) Determining the contribution of mitochondrial dysfunction in ALS pathogenesis
 \$118,750 1/1/2010 12/31/2010 Year 3

Summary Abnormal mitochondrial morphology have consistently been reported in ALS patients and mouse models that are genetic mimics of an inherited form of ALS. Genetic methods in ALS model mice will be used to increase mitochondrial biogenesis and function, decrease production of damaging, highly reactive forms of oxygen, and increase the ability of mitochondria to control an intracellular signaling chemical (calcium). This three pronged approach should determine the contribution of specific mitochondrial dysfunctions and provide potential directions for therapies.

Hristelina Ilieva M.D., Ph.D.

(DG) Dissecting the contribution of a compromised blood brain barrier to inherited ALS
 \$60,000 7/1/2009 6/30/2010 Year 1
 \$60,000 7/1/2010 6/30/2011 Year 2
 \$60,000 7/1/2011 6/30/2012 Year 3

Summary Even though motor neurons are the cell type whose loss determines the paralysis that is the central hallmark of amyotrophic lateral sclerosis, their surrounding cells are also very important. There are three cell types largely involved in forming a barrier, called the blood brain barrier, that secures nutrients while limits the passage of noxious substances into the brain. These are the endothelial cells lining the blood vessels, the pericytes in close proximity to the endothelial cells, and the astrocytes whose processes wrap around the vessels. As leakage from the blood into the brain has been reported both in human ALS autopsy cases and multiple models of ALS disease in mice, I will determine which of those cells is responsible for disruption in the blood brain barrier, to apply genetic and viral approaches to improve the function of the blood brain barrier, and to determine how this affects onset and progression of ALS.

La Jolla - University of California

Ju Chen Ph.D.

(RG) The Role of Cypher in skeletal muscle function and disease
 \$110,000 7/1/2010 6/30/2011 Year 1
 \$110,000 7/1/2011 6/30/2012 Year 2
 \$110,000 7/1/2012 6/30/2013 Year 3

Summary Mutations in Cypher result in myofibrillar myopathy (MFM) and late-onset distal myopathy. Studies of patients with myotonic dystrophy (DM), the most common adult form of muscle dystrophy, have demonstrated impaired splicing of Cypher isoforms in skeletal muscle tissues. Cypher is also significantly down-regulated in mice exhibiting skeletal muscle atrophy. These observations suggest

that Cypher plays essential roles in skeletal muscle function and disease. A better understanding of the in vivo function of Cypher and its isoforms is key to developing potential therapies for Cypher-based MFM and potentially other myopathies. In this project, we will conduct a series of studies which will help us to understand biological functions of Cypher and its isoforms at molecular, cellular, and physiological levels and thus gain insight into mechanisms by which mutations in Cypher cause myopathies, thereby improving our general understanding of myopathy. In addition, the mouse lines will be useful as test models for potential therapies.

Stephan Lange Ph.D. (Dr. sci.nat.)

(DG) The role of novel M-band associated proteins in LGMD2J.

\$45,000 1/1/2010 12/31/2010 Year 3

Summary Several mutations at the very end of titin have been identified to cause the LGMD2J-form of muscular dystrophy. We identified two structural muscle proteins that interact with the end-region of titin. We will characterize these binding partners of titin and delineate their biological role for muscle formation and function. Ultimately, these studies may lead to a better understanding and treatment of this muscular dystrophy.

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

(RG) Modeling motor neuron degeneration in SBMA

\$110,000 7/1/2010 6/30/2011 Year 1
 \$110,000 7/1/2011 6/30/2012 Year 2
 \$110,000 7/1/2012 6/30/2013 Year 3

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

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

(RG) Modeling motor neuron degeneration in SBMA

\$95,000 7/1/2009 6/30/2010 Year 3

Summary AAV vector with one isoform of VEGF will test for therapeutic value in SBMA motor neurons.

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

(RG) Canine model of inflammatory myopathies: molecular mechanisms

\$95,772 7/1/2009 6/30/2010 Year 2
 \$99,475 7/1/2010 6/30/2011 Year 3

Summary One of the problems hindering the advancement of new treatments for human inflammatory myopathies is the lack of suitable animal models. Canine IMs occur spontaneously, have reliable clinical signs, and are treated similarly to human IMs. As in human IM patients, autoantibodies are found in canine IMs; however,

unlike in humans to date, autoantibodies in canine IMs are muscle specific. We believe that further studies of the muscle specific autoantibodies found in both CMMM and CPM may offer insight into mechanisms of development of autoimmunity in both human and canine inflammatory myopathies. Such autoantibodies are potentially also present in human IM patients. The results of this project may generate a new understanding of IMs and lead to new tools for the diagnosis and treatment of IMs in humans as well as dogs.

La Jolla - Sanford-Burnham Medical Research Institute

Rolf Bodmer Ph.D.

(RG) Cardiac dystrophin model in drosophila

\$115,178	1/1/2010	12/31/2010	Year 3
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Summary In this proposal we plan to utilize the genetic tools available in the fly model (1) to study potential downstream effectors of Dystrophin in cardiac performance and morphology, (2) to utilize the Drosophila dystrophin model to examine the ability of micro- or mini-versions of human dystrophin gene to correct the dystrophin mutant defects, and (3) to carry out genetic screens to identify new gene candidates that may contribute to the cardiomyopathies observed in human Muscular Dystrophy patients. The outcome is to elucidate the genetic pathways associated with dystrophic heart defects, and to point to potential therapeutic targets for dilated cardiomyopathy.

La Jolla - The Scripps Research Institute

Pritilekha Deka Ph.D.

(DG) Structural and mechanistic role of muscleblind protein in myotonic dystrophy

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

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

Matthew Disney Ph.D.

(RG) Targeting RNAs that cause myotonic muscular dystrophy with small molecules

\$65,087	1/1/2010	12/31/2010	Year 1
\$68,580	1/1/2011	12/31/2011	Year 2
\$71,492	1/1/2012	12/31/2012	Year 3

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

Ya Wen Liu Ph.D.

(DG) Dynamin-2 cellular function and the consequences of mutations linked to CMT

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary Genetic analysis of families with heritable Charcot Marie Tooth neuropathies has revealed several mutations in the large GTPase dynamin-2 to be associated with the disease. It is not known how these mutations change the activity of dynamin-2 and affect its function in living cells. We are experts in dynamin biochemistry and cell biology and we propose to determine exactly how these mutations affect dynamin function so that we can determine the underlying cause of disease. This information may be useful in designing therapeutic interventions to prevent the onset of disease or to lessen its severity.

Los Angeles - Cedars-Sinai Medical Center

Ronald G. Victor M.D.

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

\$311,579	1/1/2010	12/31/2010	Year 1
\$311,579	1/1/2011	12/31/2011	Year 2
\$311,579	1/1/2012	12/31/2012	Year 3

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

Los Angeles - University of California

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

(RG) Novel mechanisms to enhance utrophin expression and muscle cell function

\$120,228	7/1/2009	6/30/2010	Year 1
\$122,259	7/1/2010	6/30/2011	Year 2

\$116,170 7/1/2011 6/30/2012 Year 3

Summary Loss of functional dystrophin protein in Duchenne muscular dystrophy results in reduced muscle membrane stability, with myocyte death and muscle fibrosis. It is well-established that utrophin can replace dystrophin and stabilize the muscle cell membrane to ameliorate muscular dystrophy. Over the last ten years, intense efforts have been narrowly focused at identifying molecules that could increase utrophin mRNA transcripts; however, these efforts have not yielded any viable therapies. We have taken a broad approach to identify molecules that stabilize the sarcolemma by enhancing cell attachment. As a result, we have discovered a novel compound that improves sarcolemma stability and adhesion. Importantly, this is a FDA-approved compound that has the potential to treat DMD and other congenital muscular dystrophies. The current proposal is aimed at testing the mechanisms and feasibility of this novel approach in murine models of muscular dystrophy.

Carmen Bertoni Ph.D.

(RG) Gene correction for Duchenne muscular dystrophy mediated by modified single stranded oligonucleotide

\$120,175 7/1/2009 6/30/2010 Year 3

Summary Mouse models will be developed to study the effects of oligonucleotides on correcting dystrophin DNA for DMD.

Carmen Bertoni Ph.D.

(RG) Dystrophin gene editing strategies for Duchenne muscular dystrophy

\$136,305 7/1/2010 6/30/2011 Year 1

\$136,305 7/1/2011 6/30/2012 Year 2

\$136,305 7/1/2012 6/30/2013 Year 3

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

Bennett Novitch Ph.D.

(RG) Role of FoxP transcription factors in spinal motor neuron development

\$114,000 7/1/2009 6/30/2010 Year 2

\$114,000 7/1/2010 6/30/2011 Year 3

Summary Motor neurons (MNs) are essential for all muscle movements, and the loss of their function underlies many devastating neurodegenerative diseases such as spinal muscular atrophy, spinal bulbar muscular atrophy, and amyotrophic lateral sclerosis. While few therapies currently exist to treat these conditions, great hope has been raised by the possibility of using stem cells to replace damaged MNs and restore motor functions. To achieve this goal, it is vital to first understand how

MNs are normally formed. During embryonic development, distinct classes of MNs are generated, each dedicated to the innervation of particular groups of muscles. The recovery of movement is likely to require the regeneration of all types of MNs. However, the methods that are currently being used to make MNs from stem cells appear to generate only one of these classes, raising the following questions: Can other classes of MN be generated, and if so, will each class have a different therapeutic potential? In our preliminary work, we have found that members of the Foxp gene family are expressed by subsets of developing MNs, suggesting that these genes may contribute to the process of MN diversification. We will examine how Foxp genes participate in MN development, and test whether their function can be manipulated to create different classes of MNs from stem cells and advance the development of MN disease therapies.

James G. Tidball Ph.D.

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

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

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

Los Angeles - University of Southern California

Valerie Askanas M.D., Ph.D.

(RG) SIRT1 abnormalities in inclusion-body myositis: novel therapeutic focus

\$130,219	7/1/2009	6/30/2010	Year 1
\$130,219	7/1/2010	6/30/2011	Year 2

Summary Sporadic inclusion-body myositis (s-IBM), the most common muscle disease associated with aging, is of unknown etiology and unresolved pathogenesis. There is no treatment to slow its progression. Our long-term objective is to elucidate the IBM pathogenic cascade and develop treatments. In s-IBM, intra-muscle-fiber accumulation of Abeta, oxidative stress, endoplasmic reticulum (ER) stress, and NF-kappaB activation, appear to play important pathogenic roles. Myostatin, a negative regulator of muscle mass, is also increased in s-IBM fibers, and we have recently shown in our ER-stress-induced CHMFs model that increased activation of NF-kB increases myostatin. Thus, in s-IBM muscle fibers, NF-kappaB activation and increased myostatin, both known to cause muscle fiber atrophy, appear linked. The goal of this translational project is to study, in our established culture models of IBM that are based either on overexpression of AbetaPP or induction of ER-stress, the following: a) the influence of induced SIRT1 activation/increase, either genetically or through pharmacological agents, on the IBM-phenotype; and b) the influence of downstream mechanisms resulting from SIRT1 increase/activation.

William King Engel

(RRG) Restricted funds for inclusion body myositis

\$50	1/1/2010	12/31/2010	Year 1
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Chien-Ping Ko Ph.D.

(RG) Synaptic defects in spinal muscular atrophy
 \$88,530 7/1/2009 6/30/2010 Year 2
 \$91,872 7/1/2010 6/30/2011 Year 3

Summary The long-term goal of this research is to develop novel therapies for SMA by targeting the sites of defects contributing to motor impairments. In this investigation, we will use a mouse model mimicking type II SMA to characterize two potential sites of defects at nerve connections (synapses): nerve-muscle contacts and synaptic contacts on spinal motor neurons. We will test a novel concept that motor impairment is primarily attributed to defects in the synaptic contacts on spinal motor neurons, rather than in the nerve-muscle contacts, as suggested by the prevailing thinking. In addition, we will examine whether the activation of glial cells called microglia may contribute to synapse loss in spinal motor neurons. The research will provide a new fundamental understanding on the mechanisms of SMA and other motor neuron diseases. The findings could also lead to novel therapies by focusing on drugs that can promote formation, maintenance and function of synapses on motor neurons in the spinal cord.

Menlo Park - Stanford University**Helen M. Blau Ph.D.**

(RG) A novel mouse model for Duchenne muscular dystrophy
 \$95,000 7/1/2009 6/30/2010 Year 3

Summary Researchers will develop a new mouse model incorporating telomerase activity to more closely mimic the human DMD.

Michele Calos Ph.D.

(RG) Stem cell therapy for Duchenne muscular dystrophy
 \$100,000 7/1/2010 6/30/2011 Year 1
 \$100,000 7/1/2011 6/30/2012 Year 2

Summary Our goal is to use stem cells obtained from patients to create functional muscle fibers in muscles damaged by Duchenne muscular dystrophy (DMD). In these proof of principle experiments, stem cells will be harvested from mice with DMD. A good copy of the gene mutated in DMD (dystrophin) will be added to the cells. Then the corrected cells will be returned to the mouse, where they will home to damaged muscle and regenerate intact muscle fibers. We will use an abundant type of adult stem cell that can be purified from fat tissue and has been shown to develop into muscle (adipose-derived mesenchymal stem cells). DNA carrying the therapeutic gene will be added to the cells using safe, non-viral methods. The DNA will become a permanent part of the chromosomes and will be integrated in a biologically secure location. The corrected stem cells will be injected back into the mouse. The percentage of healthy fibers that form in various muscles will be measured. We will also work with the equivalent human stem cells, derived from fat tissue, in order to optimize conditions for work with human cells. The safety of the integration sites will be examined, and it will be verified that the cells do not cause cancer. Success in these experiments will lead to future studies in DMD dogs, preparatory to clinical trials.

Hans Katzberg M.D.

(CRTG) Effect of diaphragm pacing stimulation on sleep quality in patients with ALS

\$90,000 7/1/2009 6/30/2010 Year 2

Summary Investigators believe that studying the effects of conditioning the diaphragm on sleep quality in patients with ALS will help us better understand sleep dysfunction in this group of patients and also represents a potential novel therapeutic intervention for treating sleep dysfunction in patients with ALS and neuromuscular disease.

Lorene Marie Nelson Ph.D.

(RG) Gene-environment interactions in the etiology of ALS

\$92,107 1/1/2010 12/31/2010 Year 2

\$93,494 1/1/2011 12/31/2011 Year 3

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

William Talbot Ph.D.

(RG) A novel neuronal protein that triggers myelination in Schwann cells

\$99,000 7/1/2009 6/30/2010 Year 2

\$99,000 7/1/2010 6/30/2011 Year 3

Summary Myelin is the insulation that allows axons to rapidly transmit nervous impulses. Disruption of myelin insulation causes the symptoms of Charcot-Marie-Tooth disease and other debilitating peripheral neuropathies. Our goal is to use the zebrafish to identify new genes in the formation of myelin and their associated axons. We have recently identified a novel membrane-associated protein that is essential for cells to form myelin. Researchers will test that this protein is an axon-associated signal that triggers myelin formation by Schwann cells that when lacking may be a new model of Charcot-Marie-Tooth disease. Researchers will investigate if this protein may be signal activating another gene called Krox20 known to be disrupted in some CMT cases. These experiments will be an important step toward remyelination therapies for peripheral neuropathies and other diseases of myelin.

Merced - University of California

Wei-Chun Chin Ph.D.

(RG) Carbon nanotubes based polymer scaffolding for neuron regeneration

\$109,784 1/1/2010 12/31/2010 Year 1

\$109,784 1/1/2011 12/31/2011 Year 2

\$109,784 1/1/2012 12/31/2012 Year 3

Summary The fact that our bodies are not capable of regenerating axons and re-innervating target cells makes embryonic stem cell-based transplant therapy a feasible and promising approach for muscular dystrophy patients. Carbon

nanotubes (CNTs) with length and diameter on the scale of extracellular matrix (ECM) components such as collagen or fibronectin that can interact with stem cells and have significant influence on stem cell differentiation/fate. CNTs have been considered as an ideal candidate for scaffolds in tissue engineering applications. Other than their unique ability to conduct electricity and direct neuron growth, CNTs are also elastic with mechanical integrity, and may offer roughness on substrates for ECM absorption. These unique advantages make CNTs an ideal material for neuronal-lineage-oriented human Embryonic Stem Cells (hESCs) scaffolds. Our preliminary data have indicated PMAA (polymethacrylic acid) coated CNTs can efficiently induce neuronal differentiation. In this proposed project, we plan to develop 3D CNT-based polymer scaffolds to promote better neuron differentiation efficiency from hESCs for motor neuron repairs or neuron-transplants for neuromuscular disease.

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

Thomas Rando M.D., Ph.D.

(RG) Mechanisms of fibrosis in muscular dystrophies

\$118,750	7/1/2009	6/30/2010	Year 2
\$118,750	7/1/2010	6/30/2011	Year 3

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

Pasadena - California Institute of Technology

Frederique Murielle Ruf-Zamojski Ph.D.

(DG) In toto single-cell imaging of somitogenesis and muscle formation

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary I plan to investigate the mechanisms underlying somite and muscle formation using the zebrafish as a model. Somites are conserved structures among vertebrates and give rise to skeletal structures and muscles. The goal is to develop a complete digital reconstruction of these processes in wildtype and muscular dystrophy mutant embryos with single-cell resolution. We will combine two powerful techniques in intact living and developing embryos: 1) in toto single-cell imaging, and 2) a fliptrap genetic screen to isolate mutations and study endogenously the role of muscle-related proteins. Overall, this should give us an

unprecedented opportunity to understand how muscles are built during development, maintained throughout life, and degenerate during diseases.

Redlands - LLVARE

Xuezhong Qin Ph.D.

(RG) Therapeutic potential of PAPP-A for muscular dystrophy

\$100,111 7/1/2009 6/30/2010 Year 2

Summary Duchenne muscular dystrophy (DMD) is a lethal disease caused by a mutation in the dystrophin gene. Although the eventual cure for DMD must rely on systemic delivery of a functional dystrophin gene to all muscles, alternative strategies that improve the quality of life in DMD patients are crucial until genetic treatments are available. Insulin-like growth factors (IGFs) represent an ideal candidate for improving muscle function in DMD. However, IGF-I therapy also has limitations mainly because an effective and safe IGF-I concentration is difficult to achieve, and frequent IGF-I injections must be given to patients. Instead of increasing the amount of IGF-I in the body through IGF-I injection, we will increase the activity of IGF-I produced by patients themselves by inactivating IGF inhibitors, namely, the inhibitory IGF binding proteins (IGFBPs). This can be achieved by the use of pregnancy-associated plasma protein (PAPP)-A, which is a potent protease that specifically destroys IGFBP-2, -3, and -5. If this proof of principle is confirmed by our studies, novel therapeutic strategies to defend against the secondary symptoms of DMD could be developed in the future.

San Diego - San Diego State University Research Foundation

Sanford I. Bernstein Ph.D.

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

\$145,406 1/1/2010 12/31/2010 Year 3

Summary Our studies will test the hypotheses that: 1) specific functional defects in myosin cause hereditary Inclusion Body Myopathy type 3 (hIBM3), 2) defective myosin leads to specific cell biological and physiological abnormalities, and 3) manipulation of molecular chaperone levels or other gene products can prevent myosin dysfunction/degradation/aggregation and thereby improve muscle structure and performance.

San Francisco - California Pacific Medical Center

Robert G. Miller M.D.

(TRIG) MDA/ALS Web-Based Database

\$144,750 8/1/2008 1/31/2010 Year 3

Summary The ongoing ALS CARE program is a voluntary multicenter registry that has provided a unique source of information that may be used to improve the care of patients with ALS. The major focus of the new Web-based initiative will be to obtain long-term follow-up data and information about quality of life as well as survival. These data will be used to evaluate variations in patient care, adherence to standards of care and published practice parameters and also to help design clinical trials and epidemiological studies in ALS. An additional important focus of the Web-based ALS database will be to educate participating patients and visitors to the home page about ongoing clinical trials and clinical research studies, as well as

to present an ongoing example of the role of the MDA/ALS division in the care of ALS patients nationwide.

Robert G. Miller M.D.

(RG) Multicenter screening trial of safety and efficacy of lithium carbonate in ALS
\$130,393 3/1/2009 8/28/2010 Year 2

Summary The purpose of the study is to respond to the recent claim that a common drug, lithium carbonate, can slow the progression of Lou Gehrig's disease (ALS). Lithium carbonate appeared to have benefit in a small study of Italian patients and in a mouse model of ALS. We propose a new and efficient trial design to quickly confirm these results.

Robert G. Miller M.D.

(CRNG) Infrastructure for a small screening trial consortium
\$100,000 8/1/2009 7/31/2010 Year 2
\$100,000 8/1/2010 7/31/2011 Year 3

Summary We propose to establish a network of leading ALS centers to perform small drug trials that can screen for promising new agents in ALS. We plan to perform open label trials that can streamline the search for agents that have a large enough benefit that we can see it in small novel screening trial designs and to avoid placing too many resources into treatments that will turn out to be ineffective.

San Francisco - University of California

Douglas B. Gould Ph.D.

(RG) Interrogation of a novel genetic model of muscle-eye-brain disease.
\$144,056 1/1/2010 12/31/2010 Year 1
\$144,056 1/1/2011 12/31/2011 Year 2
\$142,802 1/1/2012 12/31/2012 Year 3

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

Santa Cruz - University of California

Manuel Ares Ph.D.

(RG) Developing a diagnostic tool for splicing misregulation in DM1 and DM2
\$100,000 7/1/2009 6/30/2010 Year 1
\$100,000 7/1/2010 6/30/2011 Year 2
\$100,000 7/1/2011 6/30/2012 Year 3

Summary The mutations that lead to myotonic dystrophy (DM1 and DM2) cause accumulation of toxic RNA in the cell nucleus, binding up splicing factors including MBNL1, and causing gene expression perturbations. Splicing is an essential step in gene expression. With expression of the toxic RNA, pre-mRNAs that normally depend on MBNL1 become abnormally spliced, leading to faulty proteins and disease. We found disease-related mis-splicing events by using splicing-sensitive

microarrays and two mouse models of DM1: one over-expresses CUG-RNA; the other does not make MBNL1. We find practically the same the splicing changes in these two genetically distinct mice, implying that CUG-RNA expression leads to loss of MBNL1 function. We observed many of these same splicing changes in human DM. We have also identified a class of genes whose expression is altered in the CUG-RNA expressing mouse but that are not dependent on MBNL1. We propose to develop a diagnostic tool to measure gene expression events affected in DM1 and DM2. We will make oligonucleotide sets for "RASL", a ligation-based nucleic acid detection method that specifically measures splicing using small amounts of RNA. This will enable the measurement of hundreds of DM-related splicing and gene expression events simultaneously, providing a rich set of biomarker data to illuminate issues of disease progression, disease severity, patient genetic background, and outcomes of therapy, while minimizing pain to patients.

South San Francisco - iPierian, Inc.

John Dimos Ph.D.

(DG) A human embryonic stem cell based model of spinal muscular atrophy.

\$60,000	7/1/2009	6/30/2010	Year 2
\$60,000	7/1/2010	6/30/2011	Year 3

Summary To study the root cause of SMA and its early progression, it is essential to observe motor neurons degenerating over time. Patient-specific induced pluripotent stem (iPS) cells, like embryonic stem cells, have the ability to form any of the body's specialized cells. Unlike embryonic stem cells, SMA-specific iPS cells contain all the genetic information that leads to SMA in patients, and can generate diseased motor neurons. Motor neurons made from healthy and SMA iPS cells can be compared in a reproducible fashion to uncover root causes of SMA. Billions of spinal cord motor neurons can be generated from iPS cells, making this system useful for discovering and testing new therapeutics. We have established SMA-specific embryonic stem cell lines previously, and our preliminary observations suggest that motor neurons made from these cells exhibit aspects of SMA. Here we propose to use a similar iPS cell-based approach to characterize fundamental events underlying SMA onset and progression, and to validate this approach for drug screening.

COLORADO

Aurora - University of Colorado Denver, AMC and DC

Kurt Beam Ph.D.

(RG) Voltage sensor for excitation-contraction coupling

\$101,146	7/1/2010	6/30/2011	Year 1
\$101,146	7/1/2011	6/30/2012	Year 2
\$101,146	7/1/2012	6/30/2013	Year 3

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

function in skeletal muscle, and provide new insights into human muscle diseases, including periodic paralyses and central core disease, which arise from mutations of the DHPR and RyR1.

William J. Betz Ph.D.

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

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

Mair E. Churchill BA, MSc, Ph.D.

(RG) Molecular characterization of mitochondrial transcription complex assembly
\$110,059 1/1/2010 12/31/2010 Year 3

Summary A mouse that lacks one of the components of the transcription machinery (called mtTFA) has many features of severe skeletal muscle degeneration, similar to those found in severe human muscular dystrophies. By obtaining three-dimensional pictures of these proteins and the machinery that they form, we will learn how these systems work. This will give us new ideas into how to overcome deficits of mitochondrial function caused by genetic disorders and disease states that result in muscular dystrophy.

Martin Gartz Hanson Ph.D.

(DG) Exploring a new Ryr1 mouse model important in neuromuscular disease
\$45,000 1/1/2010 12/31/2010 Year 3

Summary The goal of this proposal is to show that at least one gene, essential in muscle contraction, is also crucial at the neuromuscular junction (NMJ). Information obtained through a combination of electrophysiological and molecular biology methods will lead to a better understanding of the fundamental mechanisms required in the formation, connectivity and communication between the central nervous system and the muscle, which can be applied to the study and potential therapies of neuromuscular diseases.

Matthew Taylor M.D.

(RG) Genetic and cellular mechanisms of Danon disease (x-linked vacuolar myopathy)
\$92,509 1/1/2010 12/31/2010 Year 3

Summary Muscle diseases like Danon disease that are complicated by cardiac involvement carry a high morbidity and mortality. Currently, no effective therapy, other than heart transplantation, exists for Danon disease and there is an urgent need to advance understanding of this under-studied disease. This project will create human cell lines from patients with Danon disease with the goal of

understanding the basic biological mechanisms causing pathology in Danon disease. A second effort will use gene therapy to 'cure' the disease in a human cellular model and will provide a foundation for future therapeutic studies in intact animals and eventually treatments.

Boulder - University of Colorado

Kurt Beam Ph.D.

(RG) Dynamic rearrangements of the DHPR and RyR1 during excitation of normal and diseased muscle

\$100,320 7/1/2009 6/30/2010 Year 3

Summary Researchers will study how mutations in DHPR and RyR1 cause CCD, MH, and HYOPP. Experiments will determine how mutations affects the interactions between DHPR and RyR1 which affect the excitation-contraction coupling for voluntary control of skeletal muscle.

Bradley Olwin Ph.D.

(RG) Identification and characterization of a satellite stem cell

\$107,025 1/1/2010 12/31/2010 Year 3

Summary Recent data from our lab and others suggests that satellite cells are heterogeneous, suggesting that different subpopulations of cells self-renew by asymmetric cell division to maintain the satellite cell pool, while others are responsible for regenerating the muscle fibers. We plan to determine if the subpopulation of cells we have identified undergoes asymmetric cell division to replenish the quiescent satellite cell pool.

David C. Sheridan Ph.D.

(DG) Bi-directional signaling between Ca²⁺ channels in skeletal muscle EC coupling

\$45,000 7/1/2009 6/30/2010 Year 3

Summary Researchers will study how the receptors DHPR and RyR1 communicate for normal EC-coupling altered in disease.

Fort Collins - Colorado State University

Carol J. Wilusz Ph.D.

(RG) The effects of CUG-repeat expansion in myotonic dystrophy on cytokine mRNA stability

\$93,128 7/1/2009 6/30/2010 Year 3

Summary Researchers will study effect of CUG-BP on cytokines in DM1.

CONNECTICUT

New Haven - Yale University

Lynn Cooley Ph.D.

(RG) Genetic analysis of muscle function and muscle disease at the single-cell level

\$95,000 1/1/2010 12/31/2010 Year 2

\$95,000 1/1/2011 12/31/2011 Year 3

Summary We have discovered a unique *Drosophila* muscle type in which we can perform genetic experiments on single muscle cells. We plan to study the effect of several muscular dystrophy-associated genes in these fly muscles with the goal of understanding how they contribute to muscle function and coordination. Importantly, we can observe muscles in live preparations and compare muscle function in young and old flies to see how muscle function changes with age. In addition, we will study the ability of the *Drosophila* muscle to renew itself from a pool of precursor cells.

Antonio Giraldez Ph.D.

(RG) The role of microRNAs in muscle development and muscular dystrophy
\$125,304 1/1/2010 12/31/2010 Year 2
\$128,580 1/1/2011 12/31/2011 Year 3

Summary Despite their diverse genetic origins, muscular dystrophies are characterized by a failure to maintain normal muscle homeostasis. MicroRNAs (miRNAs) encode ~21nt RNAs that regulate gene expression in animals and plants, but their functions are largely unknown. Several miRNAs (miR-1, miR-206, miR-133) are conserved in all animals and are highly expressed in skeletal muscle during embryonic development and adulthood. These observations suggest the hypothesis that these and other miRNAs regulate muscle development and homeostasis in animals. To investigate the role of miRNAs during skeletal muscle development we have (i) generated zebrafish embryos mutant in the miRNA-processing enzyme (Dicer), which lack mature miRNAs, (ii) have developed antisense oligonucleotides to inhibit miRNA processing and (iii) to inhibit the regulation of individual target mRNAs. This investigation focuses on the functional analysis of microRNAs in skeletal muscle going from the experimental identification of their targets in vivo (Aim 1) to the analysis of the physiological role of individual miRNA-target interactions (Aim 2), with the long-term objective of understanding how miRNAs regulate gene expression during muscle development and disease. This project will provide fundamental knowledge to develop a therapy that aims to restore normal muscle homeostasis and growth during muscular dystrophy.

DISTRICT OF COLUMBIA

Washington - Children's National Medical Center (WDC)

Ed Connor M.D.

(SG) Antisense oligonucleotide (AON) therapies in neuromuscular diseases
\$10,000 9/1/2010 9/30/2010 Year 1

Summary Given the growing number of Antisense Oligonucleotides (AONs) entering clinical development for multiple different neuromuscular disorders, the FDA and NIH, in collaboration with the research community, are taking a proactive role in developing and promoting regulatory science for the AONs by providing a forum for neuromuscular disease stakeholders to present the 'state-of-the-science' and exchange information on issues relevant to the AONs. This two-day workshop will be divided into four sessions: 1) Toxicology and Preclinical Findings to Date, 2) Biomarkers, 3) Clinical Trial Outcomes/Endpoints, and 4) Patient Registries and Assessing Long-Term Outcomes and will be concerned with four neuromuscular disorders: 1) Duchenne Muscular Dystrophy 2) Spinal Muscular Atrophy 3) Myotonic Dystrophy and 4) Amyotrophic Lateral Sclerosis. The goal of this meeting is to allow stakeholders to explore potential pathways forward for the AONs with the eventual

goal of creating a sound scientific anchoring for neuromuscular disease clinical development programs. There will be approximately 125 attended in person and the entire meeting will be webcast live for all others to view. This meeting will provide MDA, and other stakeholders in the NMD world, with a unique opportunity to engage with the FDA and other stakeholders on issues pertaining to the most promising therapeutics for treating NMD's.

Eric Hoffman Ph.D.

(RRG) Investigation of non-hormonal steroids in muscular dystrophy therapeutics
 \$34,228 1/1/2009 12/31/2010 Year 1

Summary Glucocorticoids are standard of care for Duchenne muscular dystrophy (DMD) patients however there are significant side effects associated with their use. Therefore there is an urgent need to find compounds that have similar efficacies but no side effects. Our preliminary data shows that we can synthesize compounds that exclusively have the beneficial effects but not the side effects associated with the use of glucocorticoids in dystrophic muscle. Here we propose to test an additional novel non-hormonal steroids using robust, highly reliable in a vitro assay system. If this compound works more effectively compared to currently prescribed glucocorticoids, we will take it to pre-clinical and ultimately human clinical trials.

Eric Hoffman Ph.D.

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

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

Robert T. Leshner M.D.

(RRG) Inter-rater reliability of quantitative muscle testing vs. hand held myometry
 \$70,000 2/1/2008 6/30/2010 Year 1

Summary With increases in the numbers of experimental therapies and clinical trials in DMD, it is important to define reliable and sensitive endpoints that fulfill FDA requirements for relevance to quality of life. The aim of this study is to compare two commonly utilized pediatric strength testing measures: hand-held myometry (HHM) and the CINRG Quantitative Measurement System (CQMS). The goal is to identify a sensitive and valid tool for measuring muscle strength in children with Duchenne Muscular Dystrophy. The data obtained from this study will be used to make recommendations for strength measurement endpoints in prospective muscular dystrophy trials and provide more reliable and accurate recommendations in the clinic for strength assessment.

Robert T. Leshner M.D.

(RRG) Evaluation of limb-girdle muscular dystrophy
 \$35,000 12/1/2008 12/31/2010 Year 1

Summary Limb-girdle muscular dystrophy (LGMD) is a group of muscular dystrophies with onset of weakness in childhood or adulthood. One form of LGMD (LGMD2I, or FKRP deficiency) is caused by a defect in glycosylation (the process of adding sugar units to proteins). Our central hypothesis is that glycosylation (The process of adding sugar units to proteins) adjust the environment in muscle both by allowing flexibility of the extracellular matrix (that is the part of the tissue that provides structural support to the cells) and influencing how the cells communicate with each other. Defects in glycosylation alter both of these processes. There is evidence that patients that have defects in glycosylation may have less expression of certain proteins (TGF- α and IGF-II signaling network proteins). In order to test this hypothesis, we would like to measure the levels of these proteins. We expect the levels of the tested proteins are higher in patients with glycosylation defects as compared to patients with other muscular dystrophies. We also want to learn more about LMGD, so we can use this information in future studies

Robert T. Leshner M.D.

(RRG) Planning grant for single high dose morpholino study

\$50,000	12/1/2009	11/30/2010	Year 1
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Summary Duchenne Muscular Dystrophy (DMD) is a severely debilitating childhood neuromuscular disease that affects 1 in 3,500 newborn boys. The disease causes patients to suffer from progressive loss of muscle strength due to the absence of the dystrophin protein, making them wheelchair bound before the age of 12. Most die by early adulthood due to cardiovascular and respiratory failure. The disease is caused by a mutation in the gene coding for the dystrophin protein, which is crucial for the integrity of muscle fiber membranes. Currently there is no treatment to prevent the eventual fatal outcome. Apart from long term use of steroids, little can be done to stop the slow disease progression of DMD. Promising clinical trials are underway using anti-sense oligomers (AONs) to induce exon skipping, a novel therapeutic approach which directly addresses the lack of functional dystrophin. One such AON is a Phosphorodiamidate Morpholino Oligomer (PMO) being developed by AVI Biopharma, in Seattle, WA. Given the favorable safety profile of AVI-4658 in studies thus far, a study is being proposed that will evaluate higher doses, critical for selecting an optimal biologically active and safe dose for evaluation in Phase 2/3 clinical trials for licensure. Extensive planning is needed to drive the development of an integrated clinical operational plan and bring the study to fruition. This grant will support these efforts as well as build collaborative relationships with all stakeholders.

Terence Partridge Ph.D.

(RG) Role of the nuclear envelope in muscle satellite cell activity

\$133,000	1/31/2010	1/30/2011	Year 2
\$133,000	1/31/2011	12/31/2011	Year 3

Summary Maintenance and repair of muscle is performed by muscle satellite cells, which proliferate and differentiate into muscle at sites of damage. It has been found that some myopathies such as Emery-Dreifuss and Limb-Girdle muscular dystrophies arise because of mutations in nuclear membrane proteins such as lamins and other components of the nuclear membrane collectively called NETs. We are investigating how mutations in NETs disturb maintenance of muscle and contribute to the development of disease. Our preliminary data has identified NETs that are regulated by satellite cell activation, proliferation and differentiation. We will study how these satellite cell activities are altered when we prevent production

of these NETs. This will be performed both in tissue culture and in satellite cells grafted into living muscles of recipient mice. We have also developed a mouse line that lacks Lap2alpha and lamin, two components that, via their interactions with Rb/MyoD transcription factors, have been implicated in control of cellular proliferation. We will examine satellite cells derived from these mice to better understand proliferation and differentiation processes. Finally, we will examine how the NETs are affected in other dystrophies such as mdx and dysferlin-deficient mice. We expect these studies to shed light on disease processes, characterize novel regulatory factors involved in muscle regeneration, and identify novel targets for therapy.

Christopher Spurney M.D.

(RG) Role of Toll-like receptors in the cardiomyopathy of dystrophin deficient mice			
\$101,355	1/1/2010	12/31/2010	Year 1
\$82,308	1/1/2011	12/31/2011	Year 2
\$83,289	1/1/2012	12/31/2012	Year 3

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

FLORIDA

Gainesville - University of Florida

Tetsuo Ashizawa M.D.

(SG) The 7th International Myotonic Dystrophy Consortium Conference (IDMC7)			
\$8,000	9/1/2009	3/31/2010	Year 1

Summary The International Myotonic Dystrophy Consortium (IDMC) was established as a worldwide research consortium in 1997. Since then there have been six successful meetings, for which MDA has been a key sponsor. The seventh meeting (IDMC-7) is scheduled to be held at the University of Würzburg, in Würzburg, Germany on September 9-12, 2009. IDMC-7 shares significant common goals with MDA. The overarching goal of IDMC-7 is to bring together researchers, clinicians, other care providers, patients and family members, representatives of non-profit funding agencies and industry, and members of advocacy groups, to: 1) Enhance the open exchange of information related to DM 2) Stimulate collaborative

research among investigators worldwide to improve the understanding of the underlying pathogenic mechanisms and develop novel treatments of DM1 and DM2 patients 3) Increase the pool of students and postdoctoral fellows who wish to develop their research career in the field of DM1 and DM2 by providing an opportunity to present their work and to interact with established scientists in the field. The core meeting program consists of platform sessions on the pathomechanisms, disease models, clinical issues, molecular and symptomatic therapy, heart and DM and genetic counseling. Times for poster sessions on these topics will be strategically placed in the program. Awards will be given to students and fellows. Patient sessions will be held in German and English.

Sean Forbes Ph.D.

(DG) MRI/MRS assessment of perfusion and metabolism in dystrophic muscle

\$59,974	7/1/2010	6/30/2011	Year 1
\$59,594	7/1/2011	6/30/2012	Year 2
\$59,759	7/1/2012	6/30/2013	Year 3

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

Lucia Notterpek Ph.D.

(RG) HSP90 modulators, as potential therapeutics for Charcot-Marie-Tooth neuropathies

\$95,000	7/1/2009	6/30/2010	Year 3
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Summary Researchers will investigate the effect of HSP90 as a potential therapy for CMT1A.

Maurice Swanson Ph.D.

(RG) Regulation of MBNL3 in muscle development and disease

\$119,674	1/1/2010	12/31/2010	Year 2
\$121,911	1/1/2011	12/31/2011	Year 3

Summary Recent studies from our lab and others have indicated that myotonic dystrophy (DM) is an RNA-mediated disease caused by the expansion DNA repeats which are subsequently transcribed into pathogenic RNA repeats. These repetitive RNAs are toxic because they inhibit the activities of RNA splicing factors, the muscleblind-like (MBNL) proteins, which are essential for the development of adult tissues. While we have successfully created mouse models for DM which fail to express the Mbnl1 protein, these mice do not develop either the severe muscle

wasting characteristic of adult-onset DM or the impaired development of muscles in infants with congenital DM. This proposal is designed to test the hypothesis that another member of the Mbnl gene family, MBNL3, is sequestered in DM tissues and loss of this protein is required for disease-associated alterations in muscle development and maintenance.

Krista Vandenborne Ph.D., PT

(RG) MRI assessment of PTC124 treatment in boys with DMD

\$126,295	7/1/2009	6/30/2010	Year 1
\$129,589	7/1/2010	6/30/2011	Year 2
\$132,984	7/1/2011	6/30/2012	Year 3

Summary The goal of this project is to develop a way to observe whether or not new therapies are effective in correcting or preventing the disease process in the muscles of boys with Duchenne muscular dystrophy (DMD) without the need to take muscle biopsies. To date, the only relatively noninvasive method of monitoring the disease process is unreliable and measures blood levels of creatine kinase. We have evidence in mouse models of both DMD and limb girdle muscular dystrophy that magnetic resonance imaging can be used to track the disease progression and the effectiveness of gene therapy. We will extend our past mouse studies to boys who are participating in the phase 2A clinical trial of PTC124, a promising new drug for DMD boys with premature stop codons. The availability of a reliable, nondestructive monitoring method that can be routinely and repeatedly implemented in patients will greatly benefit future clinical trials in muscle diseases.

Lizi Wu Ph.D.

(RG) Roles of the MAML1 co-activator in myogenesis and muscular dystrophy

\$120,518	7/1/2009	6/30/2010	Year 3
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Summary Researchers will study a stem cell factor called Maml1 which has a dual role in stem cell generation and differentiation.

Miami - University of Miami School of Medicine

John Barrett Ph.D.

(RG) Long-term consequences of motor terminal stress in mouse models of familial ALS

\$85,500	7/1/2009	6/30/2010	Year 3
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Summary Researchers will determine if defective post-stress restoration of NMJ is due to inability to regenerate or to maintenance of regenerated NMJs.

Antoni Barrientos Ph.D.

(RG) Understanding the molecular basis of Leigh's syndrome associated to cytochrome C oxidase deficiency

\$94,050	7/1/2009	12/31/2010	Year 3
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Summary Cytochrome C oxidase (COX) deficiency is the most frequent cause of mitochondrial neuromyopathies in humans. Patients afflicted with these diseases present heterogeneous clinical phenotypes, including Leigh syndrome (LS), muscle weakness and encephalomyopathy. Mutations in surf1 is the most frequent cause of COX deficiency. A better understanding of COX biogenesis is essential for elucidating the molecular basis underlying this group of diseases. The main objective of the proposed research is to investigate the role of Shy1p, the yeast

homologue of Surf1p, using the yeast *Saccharomyces cerevisiae* as a model complemented with studies in cell cultures from LS patients.

Lisa Baumbach Ph.D.

(RG) XL-SMA mutations - genotype: phenotype correlations & biological implications

\$80,247	7/1/2009	6/30/2010	Year 2
\$60,491	7/1/2010	6/30/2011	Year 3

Summary Our research group has spearheaded an international effort to identify and collect families with X-linked lethal infantile spinal muscular atrophy (XL-SMA), a lethal infantile neurodegenerative disorder similar to Type I SMA, but with additional features of congenital contractures and fractures. Genetic mapping studies allowed the first identification of a candidate disease gene interval (Xp11.3-q11.2), while more recent studies narrowed the gene region. Within the last year, we have collected strong evidence supporting identification of a known gene, UBE-1, as the XL-SMA disease gene. UBE-1 catalyzes the first step in protein ubiquitination-proteasome pathway, which targets numerous proteins for degradation. Our long-term goal is to apply knowledge gained from XL-SMA disease gene discovery to both prenatal and antenatal disease detection, and implementation of therapeutic strategies. The project short-term goals are: (i) to identify and collect additional XL-SMA patients and patients with overlapping clinical phenotypes; (ii) to complete mutation screening studies in these patients; (iii) to complete preliminary investigations concerning disease pathogenesis. Identification of the causal XL-SMA disease gene allows for the first advance in many years. The combined study results will allow for the first understanding and eventual treatment of this devastating illness, as well as insight into its relationship to other motor neuron and neurodegenerative disorders.

Gavriel David Ph.D., M.D.

(RG) Calcium handling in peripheral motor axons - role in CMT disease

\$105,652	1/1/2010	12/31/2010	Year 2
\$102,669	1/1/2011	12/31/2011	Year 3

Summary In Charcot-Marie-Tooth disease type 1 (CMT1) the major symptoms of muscle weakness and atrophy arise from degeneration of motor axons, which breaks the link between motor neuron activity and muscle contraction. The reason why disruption of myelin in this disease leads to degeneration of axons is not known, but recent studies in animal models of CMT1 show that the type and spatial distribution of sodium and potassium channels undergo extensive reorganization at the sites of abnormal myelination. We discovered that physiologically-activated healthy motor axons display localized calcium elevations in the close vicinity of their nodes of Ranvier, and these calcium elevations are much more extensive in the Trembler-J mouse model of CMT1 disease. Thus we hypothesize that calcium overload contributes to axonal degeneration and motor dysfunction in CMT1A. We propose to apply state-of-the-art calcium imaging techniques to Trembler-J axons, to reveal the pathways of these pathological calcium elevations, identify the optimal agents that suppress them without interfering with normal motor function, and then test if treating Trembler-J model mice with these agents will improve their axonal survival and motor performance.

Flavia Fontanesi Ph.D.

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

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

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

Carlos Moraes Ph.D.

(RG) Increased mitochondrial biogenesis as therapy to mitochondrial disorders

\$121,224	7/1/2010	6/30/2011	Year 1
\$121,224	7/1/2011	6/30/2012	Year 2
\$121,224	7/1/2012	6/30/2013	Year 3

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

Tampa - University of South Florida

Svitlana Garbuzova-Davis Ph.D., D.Sc.

(RG) Blood-brain barrier evaluation in ALS patients

\$104,500	7/1/2009	12/31/2010	Year 2
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Summary Impairment of the blood-brain barrier (BBB), blood-spinal cord barrier (BSCB), or blood-cerebrospinal fluid barrier (BCSFB) may be involved in amyotrophic lateral sclerosis. In the spinal cord and brain of both ALS patients and animal models, immune cells were observed that may be critical in motor neuron damage. Also, compounds found in the cerebrospinal fluid of ALS patients suggest that barrier permeability may be affected. Recently, we showed disruption of the BBB/BSCB in areas of motor neuron degeneration in ALS mice. To our knowledge, no direct examinations have been undertaken to verify BBB or BSCB dysfunction in ALS patients. It is our hypothesis that the BBB and BSCB are compromised in ALS patients. These studies will determine competence of these barriers by a microscopic examination of post-mortem brain and spinal cord samples from ALS patients. We will first examine tissue from the brain and spinal cord of former ALS patients for visible barrier damage using an electron microscope. Second, we will examine the integrity of barrier vascular elements using immunohistochemical tests for various blood vessel markers. The results of this study may provide a basis for improved therapeutic strategies for the treatment of ALS.

GEORGIA

Athens - University of Georgia

Aaron M. Beedle Ph.D.

(DG) Role of muscle development/regeneration in the pathology of dystroglycanopathy

\$60,000 1/1/2010 12/31/2010 Year 2

Summary Dystroglycan is an essential link between structural components inside and outside of cells to maintain the integrity of tissues like muscle and heart. In dystroglycanopathies the bond between dystroglycan and the extracellular matrix is disturbed. Sugars that normally decorate the outer region of dystroglycan are lost so that it can't bind to proteins outside of cells, increasing susceptibility to damage. To date, mutations have been identified in five different genes as causing this disease in patients. The genes encode proteins that are known or suspected to add or regulate sugars on dystroglycan. Although the number of dystroglycanopathy patients is rising, there are no treatment options available. We have generated a new mouse model to study dystroglycanopathies and develop therapeutic options. Our early data suggest that loss of dystroglycan glycosylation in muscle development leads to severe, clinically relevant features of muscular dystrophy whereas when disruption is later (mature muscle), mice are mild or asymptomatic. Our research goal is to study mouse models with abnormal dystroglycan during development and regeneration, but restored glycosylation in mature muscle. We expect these experiments to identify a critical time frame for loss of dystroglycan glycosylation in disease severity and assess the potential for therapeutic strategies that rescue dystroglycan in mature muscle.

Atlanta - Emory University

Luciano H. Apponi Ph.D.

(DG) Mechanisms of muscle specificity in OPMD

\$60,000 1/1/2010 12/31/2010 Year 1
\$60,000 1/1/2011 12/31/2011 Year 2
\$60,000 1/1/2012 12/31/2012 Year 3

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

Gary Bassell Ph.D.

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

\$98,555	1/1/2010	12/31/2010	Year 2
\$101,555	1/1/2011	12/31/2011	Year 3

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

Michael Benatar Ph.D.

(RG) The predict and prevent amyotrophic lateral sclerosis (PAPALS) study

\$152,000	7/1/2009	6/30/2010	Year 3
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Summary Investigators will study ALS population over time to assess epidemiological markers, disease onset and biomarkers.

Michael Benatar Ph.D.

(RRG) Diffusion tensor imaging of the spinal cord in ALS

39,943	6/1/2009	12/31/2010	Year 1
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Summary Broadly speaking two types of motor nerves are affected in ALS – upper motor neurons and lower motor neurons. We currently have no good measures of the severity of upper motor neuron dysfunction. Magnetic resonance imaging techniques have been used to study the brain in ALS, but these studies have not proven useful. Since the spinal cord bears the brunt of disease in ALS, we have hypothesized that using magnetic resonance imaging techniques to examine the spinal cord may be a more useful approach. We will use a relatively new imaging technique known as diffusion tensor imaging (DTI) to examine the micro-structural properties of the spinal cord in patients with ALS.

Michael Benatar Ph.D.

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

\$175,000	7/1/2010	6/30/2011	Year 1
\$175,000	7/1/2011	6/30/2012	Year 2
\$175,000	7/1/2012	6/30/2013	Year 3

Summary Treatment options for ALS patients remain limited despite decades of research. The reasons are many and complex, but are largely reflective of the many mysteries still held by this disease. With the exception of the genetic forms of ALS, the etiology remains unknown; the diagnosis is typically made late in the disease course, at which stage therapies may offer limited prospects for slowing or

reversing disease progression. There is, therefore, an urgent need for biomarkers that might permit early diagnosis, monitoring disease progression, or tracking response to experimental therapy. The study of ALS prior to symptom onset, although a long-term undertaking, provides unique opportunities to elucidate many of the enigmatic aspects of this disease -- and we contend that such an approach is both essential and feasible. Asymptomatic individuals from familial ALS pedigrees, who are at risk for developing ALS based on their harboring a mutation in an ALS susceptibility gene, represent the only known population that could be studied prospectively. With funding from the MDA we initiated a study of this population ~2 years ago. In this follow-up project we will: (a) expand the current cohort; (b) provide uninterrupted follow-up of existing and new participants; (c) systematically evaluate a series of novel biomarkers; and (d) expand the Pre-fALS biospecimen repository for use by the ALS research community.

Michael Benatar Ph.D.

(RRG) INnovative SPIRometric Evaluation in ALS (INSPIRE ALS)

\$36,896	6/1/2010	5/31/2011	Year 1
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Summary Weakness of breathing muscles is the most significant consequence of the progressive degeneration of motor nerves that occurs in ALS. The degree of weakness of the breathing muscles is typically quantified using the forced vital capacity (FVC). Monitoring the FVC is fundamentally important to the clinical care of patients with ALS and has also found a role in evaluating the progression of disease in patients who participate in clinical trials. The FVC, however, is not without its drawbacks. Measurement of FVC requires expensive equipment as well as trained and experienced evaluators. As a result it is usually only practical to record an FVC when patients visit the clinic or a clinical trial site. Moreover, the procedures for recording FVC are physically demanding for patients with ALS. One of our long-term goals is to develop techniques for evaluating and monitoring ALS patients remotely, i.e. without the requirement to travel to a clinic or study center. Here we propose an investigation of a novel approach to measuring the strength of breathing muscles. Instead of the FVC, we propose to measure the forced expiratory volume in 6 seconds (FEV6) using a portable, inexpensive, single-patient-use device known as the Piko-6. Our goals are to demonstrate that the FEV6 can be measured as reliably as the FVC and that FEV6 is at least as good as FVC for monitoring the strength of breathing muscles in ALS patients.

Tamara Caspary Ph.D.

(RG) Defining the role of Arl13b interacting proteins in the neural-glia switch

\$148,863	7/1/2009	6/30/2010	Year 1
\$148,422	7/1/2010	6/30/2011	Year 2
\$153,642	7/1/2011	6/30/2012	Year 3

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

isolating the role of the interacting protein in the developing spinal cord, we aim to define the mechanism through which cilia are needed for normal motor neuron and oligodendrocyte development.

Jonathan D. Glass M.D.

(CRNG) Emory MDA/ALS Clinical Research Center

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary We are committed to work with other Clinical research centers to investigate treatment interventions in ALS.

Jonathan D. Glass M.D.

(RRG) Models and treatments in motor neuron diseases

\$39,943	12/1/2009	11/30/2010	Year 1
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Summary The most robust animal models of Amyotrophic Lateral Sclerosis (ALS) are organisms that express mutant human superoxide dismutase 1 (SOD1). Mutant SOD1 mice develop an age-related ALS-like disease. The cause of ALS in mice, and in humans carrying mutations in the SOD1 gene is unknown. In this application we propose to test the hypothesis that alterations in the ubiquitin/proteasome system (UPS), which is responsible for degrading abnormal proteins in cells, may underlie the cellular toxicity seen in mutant SOD1

Madhuri R. Hegde BS, MS, Ph.D.

(TRIG) Comprehensive high throughput mutation detection in genes associated with NMD

\$220,000	7/1/2009	6/30/2010	Year 1
\$220,000	7/1/2010	6/30/2011	Year 2

Summary We are designing a set of microarray-based tools that will enable the high-throughput comprehensive identification of genetic variants underlying inherited neuromuscular disorders (inherited NMDs) including Congenital Muscular Dystrophies (CMDs), Duchenne and Becker Muscular Dystrophy (DMD and BMD), Emery Dreifuss Muscular Dystrophy (EDMD), Limb-Girdle Muscular Dystrophies (LGMD), and Spinal Muscular Atrophy (SMA). This project will develop the technology and protocols leading to a novel highly sensitive, rapid and reliable diagnostic tool for enhanced molecular testing for inherited NMDs.

Augusta - Medical College of Georgia Research Institute, Inc.

Lin Mei M.D., Ph.D.

(RG) Neuromuscular junction formation

\$130,939	7/1/2009	6/30/2010	Year 1
\$130,939	7/1/2010	6/30/2011	Year 2
\$130,939	7/1/2011	6/30/2012	Year 3

Summary Muscle contraction occurs when the neurotransmitter acetylcholine binds to its receptor (AChR). This receptor needs to be concentrated in a region of muscle fibers where the nerve contacts the muscle for fast and efficient neurotransmission. Improper localization and malfunction of the AChR is implicated in neurological disorders including the congenital myasthenic syndrome and myasthenia gravis. We have recently discovered a missing link – LRP4 – in our understanding of how AChR becomes concentrated at the nerve-muscle contact. We identify LRP4 as a functional coreceptor of agrin that is necessary for agrin-induced

MuSK signaling and AChR clustering. Our research is aimed at understanding mechanisms of how the novel protein LRP4 regulates agrin/MuSK signaling and AChR clustering. These studies will contribute to developing strategies of gene therapy and of diagnostic tools for neuromuscular disorders including congenital myasthenic syndrome and myasthenia gravis.

ILLINOIS

Chicago - Johns Hopkins University

Ronald Cohn M.D.

(RG) Therapeutic potential of TGF-beta antagonism in muscular dystrophies
\$83,061 7/1/2009 6/30/2010 Year 3

Summary Losartan, a TGF-B blocker will be tested for therapeutic importance in DMD and possible forms of LGMD.

Brett Morrison M.D., Ph.D.

(DG) Increasing muscle size and strength by reducing myostatin in mouse models of ALS
\$45,000 7/1/2009 6/30/2010 Year 3

Summary Studies are to use both genetic and pharmacologic methods to increase muscle size and strength in different ALS mouse models.

Chicago - Rush University Medical Center

Jingsong Zhou Ph.D.

(RG) Abnormal interactions of mitochondria and sarcoplasmic reticulum in ALS muscle
\$95,000 7/1/2009 6/30/2010 Year 3

Summary Investigators will study the alteration in mitochondrial and calcium levels during progression of ALS.

Chicago - University of Illinois

Ken-ichiro Fukuchi M.D., Ph.D.

(RG) Therapeutic delivery of anti-amyloid antibody for inclusion-body myositis
\$95,000 7/1/2009 6/30/2010 Year 3

Summary AAV vector with antibody to AB-aggregates seen in IBM patients will be tested on IBM mouse models as potential therapy.

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

(RG) Function of the alpha2/delta1 subunit in dystrophic muscle
\$108,615 7/1/2009 6/30/2010 Year 1
\$111,625 7/1/2010 6/30/2011 Year 2
\$114,707 7/1/2011 6/30/2012 Year 3

Summary The alpha2/delta1 subunit is a membrane protein that forms part of a calcium channel complex necessary to initiate contraction in adult muscle. However, we recently found new surprising evidence indicating that alpha2/delta1 is not associated with this calcium channel complex in myoblasts and that it has novel and previously unidentified roles. Our newest evidence indicates that the alpha2/delta1 subunit is involved in detecting and transmitting extracellular signals to the interior

of muscle precursor cells. Muscle repair is essential for maintaining and improving function in patients with Duchenne muscular dystrophy. Central to the repair mechanism is the activation of satellite cells. The goal of this project is to determine the role of the alpha2/delta1 subunit in the activation and migration of satellite cells in muscle from the mdx mouse model of Duchenne muscular dystrophy.

Matthew N. Meriggioli M.D.

(RG) Antigen-specific immunoregulation of experimental myasthenia gravis

\$111,597	7/1/2009	6/30/2010	Year 1
\$111,962	7/1/2010	6/30/2011	Year 2
\$114,328	7/1/2011	6/30/2012	Year 3

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

JianRong Sheng Ph.D.

(DG) Immune regulation of experimental autoimmune myasthenia gravis

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

Summary In myasthenia gravis (MG), specific immune cells, B cells, produce antibodies (with the help of T cells) that bind to the muscle and produce muscle damage and weakness. Current treatments for MG suppress the immune system as a whole. Unfortunately, these treatments are not focused and cause widespread changes in immune function, increasing the risk for infections and malignancy. We have used a particular growth factor (GM-CSF) to induce a specialized type of regulatory immune cell (regulatory T cell) in mice with experimental MG, and have successfully suppressed MG in these mice. It appears that this treatment leads to suppression of the immune cells (T cells and B cells). Since B cells play a more direct role in MG, we now propose to examine methods of generating "regulatory B cells" using GM-CSF. We will further explore the potential of these cells as a treatment for MG in mice. The information gained from these studies may help to develop a better treatment for human MG that harnesses the immune system's own regulatory network to re-establish "immune harmony," eliminating the need for chronic immunosuppression.

Chicago - The University of Chicago

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

(RG) NIH-RFA nuclear membrane protein interaction in muscle disease

\$99,980 7/1/2009 6/30/2010 Year 3

Summary Mutations in genes that encode proteins of the nuclear membrane are a common cause of muscular dystrophy. My laboratory is interesting in determining how changing the nuclear membrane leads to muscle disease.

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

(RG) Ferlin proteins in receptor recycling, membrane repair and muscular dystrophy

\$106,796 1/1/2010 12/31/2010 Year 1

\$106,796 1/1/2011 12/31/2011 Year 2

\$106,796 1/1/2012 12/31/2012 Year 3

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

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

(SG) Ottawa Conference on New Directions in Biology and Disease of Skeletal Muscle

\$20,000 4/1/2010 10/30/2010 Year 1

Summary This proposal seeks partial support for an international symposium on "New Directions in Biology and Disease of Skeletal Muscle," to be held May 5-8, 2010 at the Westin Ottawa, Ontario, Canada. This is the fourth meeting of this series; prior meetings were in 2004, 2006 and 2008. The first meeting in this "New Directions" series was attended by clinicians and scientists and covered pathogenesis and treatment-oriented research. A number of collaborations developed, and importantly, this meeting established groundwork that has directed clinical trial design. The third "New Directions" meeting was held in 2008 in New Orleans. This meeting grew substantially in size and received very positive feedback with strong encouragement to continue the series. The New Orleans meeting emphasized key findings suitable for translational efforts. Notably, the meeting had participation from the pharmaceutical industry to discuss target identification, high throughput screens and clinical trials. We selected Ottawa for the 2010 meeting because we are combining the New Directions meeting with the Ottawa Neuromuscular Conference, reflecting the large group of neuromuscular researchers in Canada and their participation in the prior New Directions meetings. We anticipate a similarly sized meeting to the New Orleans meeting (300 participants). This meeting marks the progress in the field and the evolution from basic to translational science.

Raymond Philip Roos M.D.

(RG) Transgenic mouse studies and therapeutic directions in ALS

\$85,500 7/1/2009 6/30/2010 Year 3

Summary ALS is a neurodegenerative disease characterized by the selective loss of motor neurons (MNs). Approximately 10% of ALS cases are familial (known as FALS), and ~25% of FALS cases are caused by mutations in Cu/Zn superoxide dismutase type 1 (SOD1). We plan to use transgenic mice that express mutant SOD1 to clarify the reasons for MN cell death. We will examine whether decreasing MTSOD1 expression prior to disease onset delays its onset or slows its duration, whether increasing the normal form of SOD1 expression accelerates disease, and whether stopping a normal cellular response to misfolded proteins slows disease onset. These studies may provide insights into MN death and new treatment directions.

Kamal Sharma Ph.D.

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

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

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

Maywood - Loyola University Chicago Stritch School of Medicine

Renzhi Han Ph.D.

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

\$135,000	7/1/2010	6/30/2011	Year 1
\$135,000	7/1/2011	6/30/2012	Year 2
\$135,000	7/1/2012	6/30/2013	Year 3

Summary A subset of muscular dystrophy referred to as dysferlinopathies are caused by mutations in the gene encoding dysferlin, a protein shown to play an important role in the membrane repair process in striated muscles. At present, no therapy exists for dysferlinopathies. Our long term goal is to design a therapy for dysferlinopathies. Loss of dysferlin in skeletal muscle results in prominent muscle inflammation and muscle wasting. However, questions remain concerning what causes muscle membrane injury and whether immunological attack plays an active

role in muscle injury in the absence of dysferlin. Resolving these questions may provide clues for the development of effective treatment strategies. This project focuses on exploring the effect of interfering the complement system on the muscle pathology associated with dysferlin deficiency. The overall results of these experiments will advance our understanding of the pathological mechanism underlying dysferlinopathies. Future studies will use this information for the development of therapeutic strategies to treat dysferlinopathies.

INDIANA

Indianapolis - Indiana University (Indianapolis)

Elliot J. Androphy M.D.

(RG) Vesicular transport factor interacts with SMN and the pathogenesis of SMA
 \$104,856 1/1/2010 12/31/2010 Year 3

Summary Our goal is to uncover the specialized functions of the SMN protein in the motor neuron, loss of which lead to progressive muscle weakness. We have identified a novel SMN binding protein that is involved in transport of proteins in other parts of the cell and hypothesize its interaction with SMN may be used for a different type of transport in motor neurons.

Tatiana M. Foroud Ph.D.

(TRIG) International Spinal Muscular Atrophy Patient Registry
 \$34,105 7/1/2009 6/30/2010 Year 2
 \$35,129 7/1/2010 6/30/2011 Year 3

Summary The International Spinal Muscular Atrophy Patient Registry was founded in 1986 to provide a link between patients and families interested in participating in research and researchers interested in studying SMA. The registry currently contains 1,568 families with 18,221 family members. The purpose of this application is to seek funds to continue the expansion and development of the International Spinal Muscular Atrophy Patient Registry and to: Implement new initiatives to identify, recruit and retain SMA patients and families. Collect more extensive and uniform clinical and epidemiological data. Track research study participation including enrollment, withdrawal, and completion. Establish international collaborations to develop and maintain a database of uniform, de-identified data in collaboration with the organization, Translational Research in Europe for the Assessment and Treatment of Neuromuscular Diseases. Expand awareness and use of the registry by SMA researchers.

William Groh M.D.

(RG) Continued follow-up in the registry of arrhythmias in myotonic dystrophy type 1
 \$94,588 1/1/2010 12/31/2010 Year 1
 \$90,440 1/1/2011 12/31/2011 Year 2
 \$89,751 1/1/2012 12/31/2012 Year 3

Summary In 1997 we initiated recruitment into a clinical Registry of Arrhythmias in Myotonic Dystrophy at 23 MDA clinics throughout the U.S. to gather medical, genetic, and heart data on patients with the neuromuscular disease, myotonic dystrophy type 1. We enrolled 406 patients with myotonic dystrophy type 1. Although our study was initiated to look primarily at heart issues, our careful collection of patient information has allowed us to understand better the lives of

those affected by DM1. Our current goal is continue monitoring these patients to determine their overall outcomes. Our data will provide a knowledge base to assess and compare outcomes of current practices to future interventions or therapies in the myotonic dystrophy population.

West Lafayette - Purdue University

Shihuan Kuang Ph. D.

(RG) Enhancing satellite cell self-renewal in dystrophic muscle

\$112,301	1/1/2010	12/31/2010	Year 2
\$111,000	1/1/2011	12/31/2011	Year 3

Summary Growth and repair of skeletal muscle depend on a sustained supply of satellite cells, whose number is normally maintained through a self-renewal process. In Duchenne muscular dystrophy, a lethal degenerative disease that affects some 250,000 Americans, even normal bouts exercise cause extensive muscle damage, accompanied by rapid exhaustion of satellite cells and failure in the repair of damaged muscles. In this project, we aim to investigate why satellite cells are depleted, and to explore novel approaches to restore satellite cells and the intrinsic regenerative capacity of dystrophic muscles. We address these questions using a mouse model (mdx) of Duchenne muscular dystrophy. First, the self-renewable satellite cells will be genetically tagged to assess whether they are depleted in mdx mouse. Meanwhile, mdx satellite cells will be cultured to determine whether their self-renewal capacity is impaired. Next, we will investigate whether depletion of satellite cells in mdx is due to disruption of signaling events controlling stem cell self-renewal. In this regard, the 'Notch' signaling will be examined using a transgenic mouse in which all the Notch positive cells exhibit green fluorescence. Lastly, we will genetically boost the Notch signaling in mdx mice and investigate whether stem cell self-renewal and muscle regeneration will be improved. These studies may lead to potential therapeutic approaches to improve muscle repair and delay disease onset of muscular dystrophies.

IOWA

Iowa City - The State University of Iowa Foundation

Kevin Peter Campbell Ph.D.

(RRG) Molecular pathogenesis of UCMD/Bethlem Myopathy

\$21,449	4/1/2010	3/31/2011	Year 1
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Summary The long term goal of this project is to determine the disease mechanism of Ullrich's Congenital Muscular Dystrophy (UCMD)/Bethlem Myopathy which is caused by mutations in the collagen VI protein. This project focuses on understanding the pathogenesis of UCMD/Bethlem Myopathy through further exploration of Ullrich CMD. Patient fibroblasts with collagen VI mutations will be used to understand the molecular pathogenesis of collagen VI mutations, how the mutations will lead to disease, and to test therapeutic strategies. In addition, a mouse model with a collagen VI mutation will be created to study the disease mechanism and to test therapeutic strategies. The overall results of these studies will lead to an understanding of the disease mechanism of Ullrich's Congenital Muscular Dystrophy/Bethlem Myopathy, collagen VI mutations, and will give insight into the development of treatment strategies for UCMD/Bethlem Myopathy.

Kevin Peter Campbell Ph.D.

(RG) Molecular basis of glycosylation-deficient muscular dystrophy

\$158,623	1/1/2010	12/31/2010	Year 1
\$158,623	1/1/2011	12/31/2011	Year 2
\$158,623	1/1/2012	12/31/2012	Year 3

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

Shawn Flanagan Ph.D.

(RG) Role of reactive oxygen species in ALS-associated mutant SOD1 toxicity

\$52,857	1/1/2009	6/30/2010	Year 3
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Summary It is known that mutant SOD1 in familial ALS causes an increase in reactive oxygen species (ROS) known to be detrimental to tissues. We will attempt to learn the source of the increased ROS in order to develop therapies.

Erik Paul Rader Ph.D.

(DG) Efficacy of LARGE as a therapeutic strategy for limb-girdle muscular dystrophy

\$45,000	1/1/2010	12/31/2010	Year 3
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Summary I will test whether overexpression of LARGE anchors the dystroglycan complex to the surface membrane and minimizes the pathology in mouse models of sarcoglycan deficiency. I will study skeletal muscle structure and function following LARGE overexpression in mice that lack alpha-, beta-, gamma-, or delta-sarcoglycan. The results will shed light on the molecular pathogenesis of sarcoglycanopathy but also on the therapeutic potential of pharmacological strategies that modulate expression and/or activity of LARGE.

KENTUCKY**Lexington - University of Kentucky Research Foundation****Stefan Stamm Ph.D.**

(RG) Promotion of exon 7 inclusion to treat spinal muscular atrophy

\$129,828	7/1/2009	6/30/2010	Year 1
\$129,828	7/1/2010	6/30/2011	Year 2
\$129,828	7/1/2011	6/30/2012	Year 3

Summary Spinal muscular atrophy (SMA) is caused by the loss of one gene, SMN1. All patients with SMA have an almost identical 'backup' gene, SMN2. The problem is that the body cannot put a critical piece of genetic information into the readout of the SMN2 gene. As a result, the SMN2 gene cannot substitute the loss of SMN1. However, the program to include this genetic information can be stimulated, for example by drugs like valproic acid that are now in clinical testing. It is not clear how these drugs affect the cellular program, known as alternative splice site

selection. This is a problem, because it prevents a rational improvement of therapies. The project aims to understand how this stimulation works and how drugs promote the correct use of the SMN2 gene. We will also test a new class of chemical compounds at hand that would selectively promote the inclusion of the missing critical piece of genetic information.

Louisville - University of Louisville Research Foundation, Inc.

Ashok Kumar Ph.D.

(RG) Therapeutic targeting of matrix metalloproteinases in muscular dystrophy

\$116,402 7/1/2010 6/30/2011 Year 1

\$116,402 7/1/2011 6/30/2012 Year 2

\$116,402 7/1/2012 6/30/2013 Year 3

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

LOUISIANA

Baton Rouge - Louisiana State University

Eric A. First Ph.D.

(RG) Understanding the connection between the protein synthesis and Charcot-Marie-Tooth disorder

\$110,000 1/1/2009 6/30/2010 Year 3

Summary Researchers will study mutations in the genes causing CMT-2D and DI-CMTC, two types of Charcot-Marie-Tooth (CMT) disorder.

MAINE

Bar Harbor - The Jackson Laboratory

Laurent Bogdanik Ph.D.

(DG) A new mouse model for agrin-related congenital myasthenic syndromes.

\$60,000 7/1/2009 6/30/2010 Year 2

\$60,000 7/1/2010 6/30/2011 Year 3

Summary We have found a new mouse mutation causing a severe myasthenia that will serve as an animal model for human diseases in which the function of the proteins MuSK and rapsyn are altered by genetic mutations or autoimmunity. This mutation affects a protein called agrin that acts upstream of both MuSK and

rapsyn; it should, therefore, recapitulate the features of both MuSK and rapsyn mutations. It also more closely resembles human disease-causing mutations than the previous mouse mutations available for agrin, MuSK and rapsyn. We will complete the characterization of this mutation so that it can be used to study human myasthenia mechanisms and treatments. Our finding that agrin mutation causes a myasthenia suggests that agrin itself is involved in human diseases; further, the mutation seems to modify agrin interactions with other, undetermined proteins. We will identify these new partners of agrin, which could be responsible for orphan myasthenic syndromes in humans. Using this new myasthenia model, we will pursue a pharmacological study to determine how neurotransmitter release impacts disease progression. Under certain circumstances, neurotransmitter release can disrupt neuromuscular junctions, an effect that is counteracted by agrin. However, myasthenia therapies often increase neurotransmitter activity. We will test whether these treatments may actually worsen the defects caused by deficiencies in agrin, MuSK or rapsyn.

Yi Luo Ph.D.

(DG) Roles of Notch signaling during skeletal muscle development and function

\$60,000	7/1/2009	6/30/2010	Year 1
\$60,000	7/1/2010	6/30/2011	Year 2
\$60,000	7/1/2011	6/30/2012	Year 3

Summary The Notch signaling pathway is an evolutionarily conserved cell signaling system that controls cell fate determination. It is essential for proper embryonic development in organisms as diverse as insects, nematodes and mammals. The core components of the Notch signaling pathway are Notch receptors and their corresponding ligands. In mammals, four Notch receptors have been described: Notch1, 2, 3 and 4. Five genes encoding ligands for the Notch receptors have been described: Jag1, Jag2, Dll1, Dll3 and Dll4. Despite the fact that previous work has revealed a role for Notch signaling in regulating myogenesis and skeletal muscle satellite cell activation, no progressive Notch signaling-dependent myopathy model has been described. We have found that mice doubly heterozygous for mutations of the Notch ligands Dll1 and Jag2 exhibit a progressive postnatal myopathy that preferentially affects back muscles. This model provides a valuable new resource for studying the role of Notch signaling during skeletal muscle function in mammals. Here we propose to carry out a careful analysis of the postnatal myopathy in Dll1/Jag2+/- mice and also determine the roles of Dll1 and Jag2 genes during skeletal muscle development and function. This work will provide new insights into the biology of muscle development, physiology and regeneration, and could lead to the identification of novel therapeutic targets and development of new treatments for skeletal muscle disorders.

MARYLAND

Baltimore - Kennedy Krieger, Inc.

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

(RG) Reducing fibrosis in muscular dystrophy

\$103,532	1/1/2010	12/31/2010	Year 3
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Summary The purpose of the proposed research is to determine the mechanisms by which various growth factors influence muscle fibrosis and to attempt to reduce fibrosis through modulation of these factors. Identifying the cells involved with

fibrosis formation and attempting to reverse fibrosis by regulating these cells with a myostatin inhibitor in animal models of muscular dystrophy. Since a variety of myostatin inhibitors are in pharmaceutical development, information regarding their anti-fibrotic effects can be put into clinical practice imminently.

Baltimore - Johns Hopkins University School of Medicine

Elizabeth H. Chen Ph.D.

(RG) Regulation of WASP-interacting protein during myoblast fusion
\$83,600 1/1/2010 12/31/2010 Year 3

Summary This project will investigate the regulation of an important factor required for myoblast fusion. These studies may lead to insights into certain muscle diseases and effective modulation of the fusion process in therapeutic settings in the future.

John W. Griffin M.D.

(RG) New strategies for axonal protection in murine models of CMT type 1
\$150,240 1/1/2010 12/31/2010 Year 1
\$150,240 1/1/2011 12/31/2011 Year 2
\$150,240 1/1/2012 12/31/2012 Year 3

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

Se-Jin Lee M.D., Ph.D.

(RG) Identification of new modulators of muscle growth
\$109,250 7/1/2009 6/30/2010 Year 2
\$109,250 7/1/2010 6/30/2011 Year 3

Summary We previously identified myostatin as a secreted protein that normally acts to suppress muscle growth. Considerable effort has been directed at developing drugs capable of blocking myostatin activity, as such drugs could have widespread applications for treating patients with muscle degenerative diseases. In recent work, we have demonstrated the existence of other signaling proteins that cooperate with myostatin to block muscle growth. The goal of this proposal is to begin to identify these other proteins and their receptors, as such proteins would be attractive targets for drug development.

Maureen A. Lefton-Greif Ph.D.

(RG) Coordination of respiration with deglutition/phonation in DMD and SMA
\$82,606 1/1/2009 6/30/2010 Year 2

Summary We propose to study an innovative approach to assessment of dysphagia in children with DMD and SMA by using a respirodeglutometer (RDG) to record several channels of biophysical responses (nasal airflow, laryngeal motion and pharyngeal sound) during swallows. RDG may provide a practical means for early detection and characterization of dysphagia, reducing the associated pulmonary morbidities, for children with DMD, SMA and other neurologic disorder

Brett Morrison M.D., Ph.D.

(RG) Astroglial monocarboxylate transporter (MCT) pathway in ALS

\$100,001	1/1/2010	12/31/2010	Year 1
\$100,001	1/1/2011	12/31/2011	Year 2
\$100,001	1/1/2012	12/31/2012	Year 3

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

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

(RRG) Robert Packard Center for ALS Research (Wings Over Wall Street 2008)

\$176,583	6/1/2009	5/31/2010	Year 1
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Summary MDA funding received (as directed by Wings Over Wall Street) will be used to help fund two (2) research projects through the Robert Packard Center for ALS Research. Each project is affiliated with a Hopkins-based researcher, who will participate in the Packard Center's collaborative process, and has been reviewed and approved by the Center's Scientific Advisors. Any additional funding required by these projects beyond that awarded by MDA's designated grant will be covered by the Packard Center. Money received from MDA/Wings Over Wall Street will not be used to support Dr. Rothstein or his lab.

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

(RG) Small molecule induced astroglialogenesis

\$123,095	1/1/2010	12/31/2010	Year 1
\$123,095	1/1/2011	12/31/2011	Year 2
\$123,095	1/1/2012	12/31/2012	Year 3

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

Hiromi Sesaki Ph.D.

(RG) Understanding the biochemical basis of CMT type 2A

\$133,605	1/1/2010	12/31/2010	Year 3
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Summary We are investigating the functions of mitofusin 2 using the yeast homolog Fzo1p at the molecular level. We have shown that Fzo1 directly mediates mitochondrial fusion using purified proteins. In this proposal, we will determine the molecular function of mitofusin 2/Fzo1p in mitochondrial fusion. Outcomes of this proposal will significantly enhance our understanding of the biochemical role of mitofusin 2/Fzo1p, and provide novel insights into the pathogenesis of Charcot-Marie-Tooth neuropathy type 2A.

Shanthini Sockanathan Ph.D.

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

\$121,026	7/1/2010	6/30/2011	Year 1
\$112,942	7/1/2011	6/30/2012	Year 2
\$113,864	7/1/2012	6/30/2013	Year 3

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

Baltimore - University of Maryland

Robert J. Bloch Ph.D.

(RG) Intermediate filament proteins in skeletal muscle

\$114,000	1/1/2010	12/31/2010	Year 3
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Summary We are studying the special roles of two filaments, desmin and keratin, both termed "intermediate", to learn how they work together to link the contractile structures to each other and to dystrophin at the cell membrane, and how the absence of these proteins, alone or together, compromises the structure and function of skeletal muscle.

Robert J. Bloch Ph.D.

(RG) Mu-crystallin and the pathogenesis of FSHD

\$112,971	1/1/2010	12/31/2010	Year 1
\$112,971	1/1/2011	12/31/2011	Year 2

Summary Facioscapulohumeral Muscular Dystrophy (FSHD) is caused by changes at the end of chromosome 4q in man, but no specific genes on chromosome 4 have been directly tied to the changes that occur in FSHD. My laboratory has been identifying the proteins in FSHD muscle cells that distinguish them from healthy controls and from other dystrophic muscles. We identified one such protein, called mu-crystallin. As mu-crystallin is also linked to retinal and hearing defects, which are also common to patients with FSHD, we will extend our studies of mu-crystallin to learn how closely it is linked to FSHD, to test an animal model in which mu-crystallin is expressed in mouse muscle at levels similar to those we have seen in human samples, and to examine other mouse models, created by other laboratories as part of their studies of FSHD, to learn if mu-crystallin is altered.

Diana Ford Ph.D.

(DG) Obscurin signaling through RhoA in skeletal muscle

\$60,000 1/1/2010 12/31/2010 Year 2

\$60,000 1/1/2011 12/31/2011 Year 3

Summary Dystrophic muscle is much more susceptible to injury, so it is important to determine not only what the mechanisms underlying muscle repair and regeneration are, but also how the missing components in dystrophic muscle lead to perturbations of these recovery pathways. The primary goal of the proposed research is to examine the role of rhoA in these pathways, in particular through its interaction with obscurin. Obscurin is a giant scaffolding protein in muscle that not only serves a structural role, but also mediates signaling. Concentrated around each contractile unit of muscle, obscurin is well positioned to sense force transmission. RhoA is a small signaling protein that has been shown to mediate muscle growth and differentiation and that, I have shown, may be activated by obscurin when muscle contracts forcefully. Here I propose to study how obscurin and rhoA regulate muscle fibers, and how their pathways are altered in dystrophic muscle.

Patrick W. Reed Ph.D.

(DG) Analysis of changes in the proteome in facioscapulohumeral muscular dystrophy

\$45,000 7/1/2009 6/30/2010 Year 3

Summary Researchers will attempt to discover gene products altered in FSHD muscle to determine how such a change may effect FSHD.

Bethesda - Federation of American Societies for Experimental Biology

Thomas Rando M.D., Ph.D.

(SG) FASEB Conference: Skeletal Muscle Satellite and Stem Cells

\$7,500 7/1/2010 7/31/2010 Year 1

Summary The conference is a FASEB summer conference that will allow scientists from around the world to present and discuss their latest findings on satellite and stem cells in postnatal skeletal muscle. In mature muscle, satellite cells are the primary stem cells, comprise a small percentage of the nuclei in the tissue, and exist in a quiescent state. In response to signals for postnatal growth, hypertrophy, and regeneration, satellite cells undergo proliferative expansion to generate progeny that participate in the growth and repair of skeletal muscle. This proposal is for a FASEB Conference to be held in the summer of 2010 and will be the sixth conference focused on muscle satellite cells. The session topics for the proposed conference will include topics that are at the cutting edge of stem cell research: developmental origins of satellite cells; satellite cell heterogeneity; satellite cell quiescence and activation; satellite cell self-renewal; muscle stem cell niche; pluripotentiality of muscle stem cells; molecular control of myogenic lineage progression; muscle progenitor cell differentiation and fusion; aging of muscle stem cells; muscle progenitors in growth and hypertrophy; cellular therapies in disease and atrophy. We believe that this FASEB conference has become the premier meeting for muscle stem cell biology, intersecting with related topics in general stem cell biology, muscle development, and muscle diseases.

Melissa Spencer Ph.D.

(SG) FASEB Summer Research Conference on Biology of the Calpains in Health and Disease

\$5,000 7/1/2010 7/31/2010 Year 1

Summary A FASEB summer research conference is planned for July 2010 entitled "Biology of Calpains in Health and Disease". This is the fifth such meeting focusing on calpains and it will be held in Carefree, Arizona. The meeting this year will have two main emphases; 1) understanding the biochemical properties of calpains and 2) understanding the role of calpains in various disease processes. This conference is significant in that it brings together an international group of experts who are interested in understanding the role of these proteins in different physiological and disease processes and through these interactions, propels us to a deeper and more sophisticated understanding of how to regulate their activity. Three years have passed since the last meeting on calpain and substantial progress on the genomics, structure activation and roles in disease have been made. The proposed program will provide the forum by which the momentum in this field may be maintained.

Rockville - Food and Drug Administration

Jakob Reiser Ph.D.

(RG) Remote therapeutic gene delivery for SMA

\$80,837	9/1/2009	8/31/2010	Year 1
\$87,504	9/1/2010	8/31/2011	Year 2
\$81,771	9/1/2011	8/30/2012	Year 3

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

Rockville - Validus BioPharma, Inc.

Erica K.M. Reeves Ph.D.

(MVP) Preclinical validation of a non-hormonal steroid for Duchenne muscular dystrophy

\$360,000	1/12/2010	12/1/2010	Year 1
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Summary We have discovered a family of compounds that have steroidal backbones but which lack both metabolic and hormonal activity. Our compounds are non-hormonal and showed significant anti-inflammatory properties (e.g., NF-kB activity) in skeletal muscle cells while lacking hormonal activity. Preliminary data shows that these non-hormonal steroids are dramatically effective in the mouse model of muscular dystrophy with improved body weight, skeletal muscle function and histology. We will prepare variants that fulfill our objectives of safety, efficacy, and satisfactory pharmacokinetics. We will prepare compounds, demonstrate in vitro efficacy and safety, test for pharmacokinetics with a variety of in vitro and in vivo assays, and then demonstrate activity in the mouse model for optimal compounds. The best one or two compounds will be declared candidates and pre-IND work will be completed in preparation for clinical trial in Duchenne children.

MASSACHUSETTS

Boston - Beth Israel Deaconess Medical Center

Zolt Arany M.D. Ph.D.

(RG) PGC-1 coactivators and angiogenesis in muscular dystrophy

\$118,232 7/1/2010 6/30/2011 Year 1

\$116,978 7/1/2011 6/30/2012 Year 2

\$116,978 7/1/2012 6/30/2013 Year 3

Summary It has become increasingly clear that skeletal muscle metabolism plays a critical role both in the onset of, and resistance to Duchenne and other muscular dystrophies. Our lab studies a small group of molecules, the PGC-1s, which powerfully regulate broad metabolic programs in various tissues. In skeletal muscle, these molecules markedly alleviate muscle degeneration in mdx mice, a model of DMD. How this happens remains unclear. Recently, we have shown that PGC-1s control a new and powerful pathway that induces the formation of new blood vessels in muscle. We therefore propose here the hypothesis that these molecules alleviate muscle degeneration and atrophy by boosting microvascular density and signaling. The hypothesis will be investigated here using a variety of molecular and genetic means, using the mdx mouse as a model of DMD. Understanding precisely how PGC-1s protect skeletal muscle against the ravages of dystrophy may lead to novel therapeutic approaches for these devastating diseases.

Boston - Boston Medical Center

Kimberly Kafadar Long Ph.D.

(DG) Mechanisms of Sca-1 regulation by TGFb

\$60,000 7/1/2009 6/30/2010 Year 2

Summary Whether induced by exercise, trauma, or disease, the regenerative ability of skeletal muscle is largely dependent on satellite cells, a population of stem cells that resides in skeletal muscle along the myofibers. In response to growth stimuli, satellite cells are induced to proliferate, differentiate, and fuse to form new myofibers or fuse into existing myofibers. We are interested in how Sca-1, a protein present in numerous stem cell populations, affects muscle growth and regeneration. Transforming growth factor-beta 1 (TGF-b1) is highly upregulated in response to muscle injury. TGF-b1 dysregulation in multiple muscular dystrophies results in extensive connective tissue deposition. This increase in fibrosis greatly inhibits the full recovery of the muscle. Modulation of TGF-b1 in dystrophic patients may be of great therapeutic value. We have shown that TGF-b1 is a novel negative regulator of Sca-1 expression in myoblasts, although the mechanism of this action is unknown. The goal of this study is to identify the pathway through which TGF-b1 regulates Sca-1 expression, including transcriptional activators of Sca-1. We believe that these studies may uncover novel therapeutic means by which to ameliorate the poor regeneration in dystrophic muscle.

Boston - Brigham and Women's Hospital, Inc.

Steven A. Greenberg M.D.

(RG) Lymphocyte maturation within muscle in the inflammatory myopathies

\$80,750 7/1/2009 6/30/2010 Year 3

Summary Researchers will study inflammatory myopathies to understand the immune system attack in these disorders.

Pavel Ivanov Ph.D.

(DG) Angiogenin and amyotrophic lateral sclerosis

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

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

Calum A. MacRae MB, ChB, Ph.D.

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

\$91,622	7/1/2009	6/30/2010	Year 1
\$90,270	7/1/2010	6/30/2011	Year 2
\$92,209	7/1/2011	6/30/2012	Year 3

Summary One of the most important complications of muscular dystrophy is involvement of the heart, which can lead to irregular heart rhythms, including heart block or sudden death, and reduced pumping strength or congestive heart failure. This cardiac involvement contributes significantly to the burden of disease, and is one of the major causes of death in many forms of muscular dystrophy. The mechanisms by which the heart is affected in muscular dystrophies remain poorly understood. Using the zebrafish, we have developed a unique set of techniques to investigate the ways in which genetic abnormalities cause various forms of heart disease. The zebrafish is transparent and so enables the study of heart abnormalities as they actually occur early in heart formation in genetically faithful models of some forms of muscular dystrophy. This zebrafish approach is very powerful since once we better understand the mechanisms of the heart disease in muscular dystrophy, we can adapt the zebrafish models of muscular dystrophy we have created to detect the heart involvement using automated microscopes. This automation will then allow us to test many thousands of chemical compounds in order to identify specific chemicals that reduce or reverse the muscular dystrophy heart defects in the zebrafish. This will rapidly identify potential new drugs for the treatment of muscular dystrophy.

Mohammad Salajegheh M.D.

(DG) The identification of antigens in inclusion body myositis

\$60,000	7/1/2009	6/30/2010	Year 2
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\$60,000 7/1/2010 6/30/2011 Year 3

Summary None of the known immune therapies have proven to be effective for treating inclusion body myositis (IBM). While both inflammatory and degenerative mechanisms are implicated in the disease process, the relationship between the two remains unclear. Studies have shown that the inflammation present in IBM muscle is antigen driven, and that it involves both cell-mediated (cytotoxic T cells) and humoral (B cells, plasma cells and antibodies) immunity. However, the nature of these antigens is unknown, and their identification will allow us to better understand the disease process and lead to the development of more specific diagnostic methods and effective therapies. Previous studies have demonstrated the feasibility of using antibodies for the identification of antigens in other inflammatory diseases. We have developed similar strategies aimed at identifying antigens in IBM, using a combination of immunologic and proteomics techniques, as well as advanced mass spectrometry. Our preliminary results have suggested that alpha B crystallin may act as an autoantigen in certain IBM patients. We propose to perform a systematic approach to further identify antigens in IBM, determine their localization within muscle and their relationship to degenerative components.

Xin Wang Ph.D.

(RG) Screening agonists of the melatonin receptor 1A against ALS

\$143,877 1/1/2010 12/31/2010 Year 1
\$143,877 1/1/2011 12/31/2011 Year 2
\$143,877 1/1/2012 12/31/2012 Year 3

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

Boston - Children's Hospital Boston

Basil T. Darras M.D.

(CRNG) Children's Hospital Boston MDA Clinical Research Center

\$100,000 8/1/2009 7/31/2010 Year 2
\$100,000 8/1/2010 7/31/2011 Year 3

Summary As a participating MDA Clinical Research Network site, we propose to conduct a natural history study that will examine the longevity of DMD patients and will take into account for the first time the molecular genetics of dystrophinopathies. We will relate clinical outcome to both molecular defect and treatment modality to assess the impact on survival and outcome of the more interventional, aggressive treatments that have been developed for our patients by medical, orthopedic, pulmonary, cardiology and gastrointestinal disciplines over the past 15-20 years.

Steven A. Greenberg M.D.

(RG) Development and screening of cellular models of dermatomyositis				
\$112,856	1/1/2010	12/31/2010	Year 1	
\$112,584	1/1/2011	12/31/2011	Year 2	
\$113,166	1/1/2012	12/31/2012	Year 3	

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

Louis Kunkel Ph.D.

(RG) Small molecule screens in dystrophin deficient zebrafish				
\$125,000	7/1/2010	6/30/2011	Year 1	
\$125,000	7/1/2011	6/30/2012	Year 2	
\$125,000	7/1/2012	6/30/2013	Year 3	

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

Maria Chiara Manzini Ph.D.

(DG) Identification of genes involved in severe CMD associated with brain defects				
\$45,000	1/1/2010	12/31/2010	Year 3	

Summary We have collected a large group of patients affected with WWS, MEB or CMD with milder eye and brain malformations. By combining clinical and genetic analyses, we will determine the impact of the known genes and identify novel genes responsible for these diseases. In the short term, this work will provide additional diagnostic tools, and in the future, it will help understand the biology of these disorders on the path to a cure.

Boston - Harvard Medical School

Marcia Haigis Ph.D.

(RG) Regulation of mitochondria by SIRT3				
\$108,422	1/1/2010	12/31/2010	Year 2	

\$113,906 1/1/2011 12/31/2011 Year 3

Summary Mitochondrial function is essential for ATP production in tissues that consume energy, such as the muscle. Dysfunction of mitochondria can lead to loss of energy production, build up of harmful oxidants and tissue decline. The goal of this study is to examine the effect of a mitochondrial enzyme, SIRT3, on mitochondrial energy production in the skeletal muscle, and to see how these parameters are affected by diet and age. SIRT3 is interesting because it is homologous to a conserved regulator of aging SIR2. In fact, the SIR2 family of proteins is hypothesized to control aspects of metabolism, obesity, and muscle mass in mammals. We have discovered that SIRT3 interacts with critical components of energy production in the mitochondria. We propose to use chemical assays to measure how SIRT3 regulates ATP and oxidants. We propose to use biological assays to measure how SIRT3 regulates muscle function by using a mouse model that lacks SIRT3 protein. We also propose to investigate how mitochondrial acetylation and SIRT3 affect a mouse model of muscular dystrophy. These experiments will provide us with new information about how mitochondrial SIR2 protein works to regulate energy in the muscle, and how it may be dysregulated during muscular dystrophies.

Andrew Lassar Ph.D.

(RG) Regulation of quiescence and differentiation of satellite cells

\$125,000	1/1/2010	12/31/2010	Year 1
\$125,000	1/1/2011	12/31/2011	Year 2
\$125,000	1/1/2012	12/31/2012	Year 3

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

Boston - Massachusetts General Hospital

Nazem Atassi M.D.

(CRTG) Comparative effectiveness in ALS symptomatic management

\$90,000	7/1/2010	6/30/2011	Year 1
\$90,000	7/1/2011	6/30/2012	Year 2

Summary We are proposing this study to determine if people with ALS can tolerate Tizanidine treatment for muscle stiffness. The long term goal is to determine the best management for a common symptom of ALS. Amyotrophic Lateral Sclerosis (ALS) is a disorder affecting the nervous system. In addition to muscle weakness, people with ALS suffer from symptoms such as stiffness, cramps, breath or swallowing difficulties, increased saliva, and depression. Potential treatments for most of these symptoms are available. The challenge is that many of

these treatments were developed for people who don't have ALS. As a result, we don't know if these treatments are tolerated, safe, or effective in people with ALS. Treatment of ALS symptoms is very important. For example, the recent use of an assisting breathing machine by people with ALS improved their sleeping, quality of life and prolonged their survival. Muscle stiffness is a common problem in ALS that usually causes slowing of movements and loss of coordination in the arms and legs. As a result ambulation and usual daily activities become difficult and often painful. We don't know the ideal dose or the effect of any of the anti-stiffness medication in ALS. Tizanidine is an approved medication that reduces muscle stiffness in other diseases. We designed this study to determine the range of dosages that people with ALS can tolerate and to obtain information about the safety and effectiveness of Tizanidine in treating muscle stiffness in ALS.

Merit Ester Cudkowicz M.D.

(RG) Validation of a new device to measure neuromuscular disease progression
 \$71,250 7/1/2009 12/31/2010 Year 3

Summary Clinicians will test ATLIS to determine isometric strength assessments in ALS patients to establish a more accurate and faster method for drug evaluations.

Merit Ester Cudkowicz M.D.

(CRNG) MDA ALS Clinical Research Network: Massachusetts General Hospital ALS Center
 \$100,000 8/1/2009 7/31/2010 Year 2
 \$100,000 8/1/2010 7/31/2011 Year 3

Summary The process of developing new drugs for ALS is particularly challenging because of relative rarity of this disorder. Low enrollment rates and high study participant withdrawal add further challenges. Due to advances in our understanding of ALS pathogenesis, there is potentially a large pipeline of therapies to bring forward for patients with ALS. The current strategy is to test one drug at a time against placebo. However, if multiple active drugs are tested against each other and then only best of that group is compared against placebo, the time required to develop an effective ALS therapy could be cut-down considerably. We propose that the MDA ALS Clinical Research Network establish a protocol and system to perform selection design studies on treatments that are ready for phase 2 testing. The Network will collaborate with regional centers to improve study enrollment and retention.

Merit Ester Cudkowicz M.D.

(RG) Trial of high fat/high calorie diet versus optimal calorie replacement in ALS
 \$231,766 7/1/2009 6/30/2010 Year 1
 \$218,525 7/1/2010 6/30/2011 Year 2

Summary Weight loss is a common and severe symptom of amyotrophic lateral sclerosis (ALS), caused both from inadequate calorie intake and an increased metabolic rate. People with ALS are generally instructed to increase their calorie intake; however, the ideal amount and type of calories has not been studied. Several studies in an animal model of motor neuron disease have shown that a high fat/high calorie diet can increase survival by as much as 38%. Mice on a high fat diet also live longer than mice fed diets consisting of high protein or high sugar. This is a phase II safety, tolerability, and preliminary efficacy trial in ALS of high fat versus high calorie versus normal diet through the MDA ALS Clinical Research

Network. Each intervention diet will be calculated based on the optimal calorie requirements needed to replace participants' measured energy expenditure. Aim 1 is to measure feasibility, compliance and serious adverse events on the three diets. Aim 2 is to measure markers of lipid metabolism and body composition before and after the study interventions. Aim 3 is to look at preliminary outcome measures including weight, BMI, the ALS Functional Rating Scale –Revised, forced vital capacity, grip strength and survival.

Anne-Marie Wills M.D.

(CRTG) Trial of high fat/high calorie diet versus optimal calorie replacement in ALS
 \$90,000 7/1/2009 6/30/2010 Year 1
 \$90,000 7/1/2010 6/30/2011 Year 2

Summary I am a board-certified neurologist at Massachusetts General Hospital specializing in amyotrophic lateral sclerosis (ALS). I will conduct a Phase II safety and feasibility study of high fat/high calorie diet versus optimal calorie replacement in ALS. People with ALS are generally instructed to increase their calorie intake; however, the ideal amount and type of calories has not been studied. Several studies in an animal model of ALS have shown that a high fat/high calorie diet can increase survival by as much as 38%. This clinical trial will measure feasibility, compliance and serious adverse events on high fat/high calorie, versus high calorie, versus normal calorie diet. We anticipate that the data from this study will aid in the design of a larger efficacy study and eventually the inclusion of dietary recommendations in an ALS practice parameter.

Boston - Tufts University School of Medicine

Yongjie Yang Ph.D.

(DG) Dysregulation of astroglial glutamate transporter EAAT2/GLT1 in ALS
 \$60,000 7/1/2009 6/30/2010 Year 2
 \$60,000 7/1/2010 6/30/2011 Year 3

Summary Glutamate-induced excitatory toxicity is one of the major pathogenic pathways in the motor neuron diseases. Severe loss of the astroglial excitatory amino acid transporter 2 (EAAT2, rodent analog GLT1), the primary transporter responsible for removing >90% of extracellular glutamate, has been found in animal models of various motor neuron diseases and postmortem patients. So far, the regulation of GLT1/EAAT2 expression and mechanisms of loss of the GLT1/EAAT2 in motor neuron diseases are very poorly understood. The proposed study aims to better understand the dysregulation mechanisms of EAAT2/GLT1 in the animal model of ALS by identifying the critical EAAT2 promoter elements and by investigating the function of a novel target protein in the regulation of EAAT2/GLT1 expression. The knowledge from these studies will help to better understand the disease pathogenesis and also provide new target for novel drug discovery.

Cambridge - ALS Therapy Development Foundation Inc.

Steven Perrin Ph.D.

(TRAC) Identification and validation of therapeutic targets for ALS clinical development
 \$6,000,000 3/1/2009 2/28/2010 Year 3

Summary The Muscular Dystrophy Association and ALS Therapy Development Institute (ALS-TDI) are collaborating on a program to comprehensively characterize

disease progression in ALS using animal models of neurodegeneration and ALS clinical samples. The unbiased approaches of genomics, proteomics, and genetics will assist in understanding biological mechanism associated with disease susceptibility, onset, and progression. An unparalleled secondary validation process will be implemented using in vivo and in vitro technologies to prioritize the most relevant molecules associated with disease biology. Proof-of-concept studies will be evaluated in murine models of neurodegenerative disease to assess the effects of putative therapeutics on surrogate markers of disease as well as survival. From these studies the ALS-TDI will develop validated therapeutic targets ready for pre-clinical and clinical development and deliver diagnostic and prognostic disease biomarkers for use in clinical applications.

Steven Perrin Ph.D.

(MVP) Development of therapeutic interventions for amyotrophic lateral sclerosis
\$2,500,000 1/1/2010 12/31/2010 Year 1

Summary The Muscular Dystrophy Association and ALS Therapy Development Institute (ALS-TDI) are collaborating on a program to comprehensively characterize disease progression in ALS using animal models of neurodegeneration and ALS clinical samples. The unbiased approaches of genomics, proteomics, and genetics will assist in understanding biological mechanism associated with disease susceptibility, onset, and progression. An unparalleled secondary validation process will be implemented using in vivo and in vitro technologies to prioritize the most relevant molecules associated with disease biology. Proof-of-concept studies will be evaluated in murine models of neurodegenerative disease to assess the effects of putative therapeutics on surrogate markers of disease as well as survival. From these studies the ALS-TDI will develop validated therapeutic targets ready for pre-clinical and clinical development and deliver diagnostic and prognostic disease biomarkers for use in clinical applications.

Cambridge - Catabasis Pharmaceuticals, Inc.

Michael Jirousek Ph.D.

(MVP) Preclinical validation of a small molecular anti-inflammatory compound for
Duchenne muscular dystrophy
\$100,000 1/1/2010 9/30/2010 Year 2

Summary Catabasis has a proprietary platform technology that enables the identification and development of small molecules that simultaneously suppress pro-inflammatory pathways and activate anti-inflammatory pathways. Catabasis is initially targeting diseases associated with muscle wasting. CAT1002, is a small molecule designed to target the macrophage and the underlying inflammation that is key to the development and persistence of the pro-inflammatory state that is found in DMD. CAT1002 is efficacious in vitro in macrophage cell lines and in vivo in mouse models of inflammation. The CAT1002 lead program is poised to enter IND enabling studies in 2H2009 and to be ready for Phase 1 human clinical testing in 3Q2010. Funds will be directed to testing the ability of CAT1002 to ameliorate the decline in muscle function in mdx mouse and to the development of an IND package to support a CAT1000 series molecule for advancement into human clinical testing in DMD.

Cambridge - Harvard College

Alexander Schier Ph.D.

(RG) Cilia in muscle and motoneuron development

\$130,040	1/1/2010	12/31/2010	Year 1
\$130,040	1/1/2011	12/31/2011	Year 2
\$130,040	1/1/2012	12/31/2012	Year 3

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

Medford - 4S3 BIOSCIENCE INC**Dustin Armstrong Ph.D.**

(MVP) Muscle targeted myotubularin 1 for treatment of congenital myotubular myopathy

\$148,138	4/1/2010	3/31/2011	Year 1
\$112,153	4/1/2011	3/31/2012	Year 2

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

Waltham - Repligen Corporation**James Rusche Ph.D.**

(MVP) Clinical development of a HDAC inhibitor for Friedreich's ataxia

\$511,534	1/1/2010	6/20/2010	Year 2
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Summary FA is caused by a decrease in the expression of the frataxin gene. HDAC inhibitors have been shown to increase the expression of frataxin in cells obtained from patients and tested in culture experiments. In studies partially funded by MDA, Repligen and collaborators have identified specific HDAC compositions and tested the effect in transgenic animal models for efficacy. These compounds can increase frataxin in mutant mice and the effect persists for some period of time after the drug is eliminated from the tissue. Preliminary toxicology in rodents and dogs indicates the compounds can achieve the efficacy dose far below a dose that is toxic. The grant proposal is to continue the development of these compounds in preclinical and clinical studies. This treatment is designed to modify the disease course by restoring the expression of the native frataxin gene.

Watertown - Boston Biomedical Research Institute

Jeffrey Boone Miller Ph.D.

(RG) MDC1A therapeutic mechanisms: model studies.

\$98,411	1/1/2010	12/31/2010	Year 2
\$100,994	1/1/2011	12/31/2011	Year 3

Summary One type of Congenital Muscular Dystrophy is caused by mutations that prevent the proper function of a protein called laminin-alpha2. One result of the loss of laminin-alpha2 function is that muscle cells die so that muscle mass and strength are greatly reduced. In model studies, we have identified methods that slow muscle cell death and ameliorate disease symptoms. These methods depend on modifying a particular molecular pathway that centers on the protein called Bax. In the studies proposed here, we will analyze additional molecular pathways that interact with and regulate Bax. By understanding how these pathways regulate Bax in diseased muscle, we will learn how loss of muscle cells occurs upon mutations of laminin-alpha2. Furthermore, it may prove possible to manipulate these additional pathways with drugs in a way that will prove therapeutically useful for congenital muscular dystrophy.

Worcester - University of Massachusetts Medical School

Michael M. Francis Ph.D.

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

\$110,000	7/1/2010	6/30/2011	Year 1
\$110,000	7/1/2011	6/30/2012	Year 2
\$110,000	7/1/2012	6/30/2013	Year 3

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

Jianhua Zhou Ph.D.

(RG) Protecting against SMN defects by stress response proteins and biological molecules

\$82,856	1/1/2009	3/31/2010	Year 3
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Summary Researchers will test if stress response proteins or small molecules may compensate for the loss of SMN protein providing a therapy for Spinal Muscular atrophy (SMA).

MICHIGAN

Ann Arbor - University of Michigan

James Dowling M.D., Ph.D.

(DG) Characterization and rescue of a murine model of myotubular myopathy
\$45,000 7/1/2009 6/30/2010 Year 3

Summary A mouse model of myotubular myopathy will be used to understand relationship of myotubularin mutations and disease.

Daniel Goldman Ph.D.

(RG) Epigenetic control of muscle activity-dependent gene expression
\$114,958 7/1/2009 6/30/2010 Year 3

Summary Muscle atrophy may be due to disruption of NMJ signaling. The role of HDACs in this pathway will be studied.

John Kim Ph.D.

(RG) The characterization of all 3'UTR isoforms for the muscle transcriptome
\$131,934 7/1/2009 6/30/2010 Year 1
\$128,919 7/1/2010 6/30/2011 Year 2
\$127,378 7/1/2011 6/30/2012 Year 3

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

Detroit - Wayne State University

John Kamholz Ph.D.

(RG) The structure and function of myelin protein zero mutations causing CMT1B
\$111,150 7/1/2009 6/30/2010 Year 2
\$111,150 7/1/2010 6/30/2011 Year 3

Summary Myelin protein zero (MPZ) is the major protein of myelin in the peripheral nervous system (PNS). Mutations in MPZ cause an inherited demyelinating neuropathy, called CMT1B, which has associated muscle weakness, sensory loss, and difficulty walking. In this study we will investigate the structure of human MPZ and several mutations that cause CMT1B. We have already determined the three dimensional structure of the normal human MPZ, and its analysis, including molecular modeling, suggests several ways that mutations could alter its structure to cause neuropathy. We will extend these studies to include analysis of the three dimensional structure and function of several mutations in MPZ that cause either early or late onset neuropathy. These studies will provide a basis for designing specific and novel treatments for this neurodegenerative disease.

Samia Ragheb Ph.D.

(RG) Baff and the B-Cell in myasthenia gravis (MG)
\$125,783 1/1/2009 3/31/2010 Year 3

Summary A newly discovered molecule, Baff, helps B-cells of the immune system to survive and multiply. Understanding this relationship will allow development of therapies for myasthenia gravis (MG)

Michael Shy M.D.

(RG) Treating the mouse model of early onset CMT1B
\$113,121 7/1/2009 6/30/2010 Year 2
\$115,876 7/1/2010 6/30/2011 Year 3

Summary Charcot Marie Tooth disease type 1B is one of the most common inherited peripheral neuropathies. Patients develop leg and arm weakness, develop balance problems and have difficulty feeling pain and temperature. Many cases of CMT1B are particularly severe and prevent children from walking until they are several years old and confine patients to wheelchairs before adulthood. We have generated a mouse model of the severe forms of CMT1B and are now trying to develop treatments for these mice that can then be tried in patients.

Michael Shy M.D.

(TRIG) North American CMT Network
\$242,877 4/1/2010 3/31/2011 Year 1

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

MINNESOTA

Minneapolis - University of Minnesota - Twin Cities

Atsushi Asakura Ph.D.

(RG) Muscle stem cell transplantation for muscular dystrophy

\$95,000	7/1/2009	6/30/2010	Year 2
\$95,000	7/1/2010	6/30/2011	Year 3

Summary Possible approaches to restoring muscle fiber degeneration in Duchenne muscular dystrophy patients include cell therapy, gene therapy or a combination of the two. Muscle contains a type of stem cell called satellite cells that give rise to newly formed muscle fibers. We demonstrate that genetically modified satellite cell-derived myoblasts, isolated from mice lacking MyoD, a muscle-specific master transcription factor, display significantly higher engraftment compared to wild-type myoblasts when injected into injured muscle. Importantly, these genetically modified myoblasts were revealed to possess remarkable resistance to cell death and increased survival after stress induction, compared to wild-type myoblasts. In addition, these genetically modified myoblasts were detected underneath the basal lamina of muscle fibers after transplantation, indicating the self-renewal property of the myoblasts. Therefore, MyoD^{-/-} myoblasts may preserve stem cell characteristics following transplantation, including resistance to cell death, efficient engraftment and contribution to satellite cells. In addition, we noticed that MyoD negatively regulates cell survival factors. Our data offer evidence for improved therapeutic stem cell transplantation for muscular dystrophy, in which suppression of MyoD in myogenic progenitors would be beneficial to therapy by providing a selective advantage for the expansion of stem cells.

John West Day M.D., Ph.D.

(CRNG) University of Minnesota Duchenne Muscular Dystrophy Clinical Research Center

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary We are eager to participate and involve the MDA DMD Clinical Research Network in approaches we are developing, which involve behavior, physical therapy, and nutritional methods, in addition to novel cell-based, protein-based and pharmacological treatments currently under development.

James M. Ervasti Ph.D.

(RG) TAT-utrophin as a protein therapy for dystrophinopathy

\$118,750	7/1/2009	6/30/2010	Year 2
\$118,750	7/1/2010	6/30/2011	Year 3

Summary These studies will continue to develop a novel protein replacement therapy in dystrophin-deficient mdx mice that may ultimately be used to stop or slow the progression of Duchenne muscular dystrophy. We will also investigate the capability of a novel targeted TAT-utrophin construct to specifically transduce dystrophic skeletal muscle, which could potentially increase efficacy, decrease the effective dosage, and further minimize the potential side effects of TAT-utrophin transduction into non-muscle tissues. Our approach complements other strategies, particularly utrophin upregulation approaches, which may potentially be combined to provide optimal benefit to patients.

Michael Koob Ph.D.

(RG) Towards gene therapy of mitochondrial disease

\$50,000	11/1/2009	10/31/2010	Year 2
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Summary We have developed technology that allows us for the first time to engineer mammalian mitochondrial genomes and reintroduce them into mouse embryos. We will use this technology to develop a gene therapy for Friedreich's

ataxia (FRDA), an autosomal recessive neurodegenerative disease caused by defects in frataxin, a nucleus-encoded mitochondrial protein. We will focus our initial efforts on correcting the molecular deficits associated with the complete loss of frataxin in yeast. This well characterized model of FRDA will enable us to systematically determine how best to efficiently express a fully functional form of frataxin from the mitochondrial genome. We will use the information and reagents developed in this initial phase of the project to engineer a set of mouse mtDNA genomes suitable for correcting deficits in a mouse model of FRDA. We will evaluate the efficiency with which these genomes compensate for the loss of the mouse nuclear frataxin gene by packaging them in mitochondria and injecting them into single cell embryos of FRDA knockout mice. This experimental system will allow us to optimize both our mitochondrial transfer technology and our mitochondrial frataxin gene constructs. We will then be in an excellent position to develop a gene therapy approach for mouse models of FRDA and to work towards adapting these therapies to treating FRDA and other mitochondrial diseases in humans.

Dawn A. Lowe Ph.D.

(RG) A bone-sparing strategy for muscular dystrophies

\$85,994	1/1/2010	12/31/2010	Year 2
\$84,516	1/1/2011	12/31/2011	Year 3

Summary The rate of bone fractures is on the rise in individuals with Duchenne Muscular Dystrophy (DMD). There are many underlying causes of bone degradation in DMD, a major one being the low stresses placed on bone by weak muscles. Because muscles and bones work together, the first aim of our project is to determine the simultaneous functional changes of these two tissues in dystrophic mice. This is essential so that treatment strategies that are optimal for both muscle and bone can be devised and evaluated. The second aim of our project is to determine the efficacy of low-level, mechanical vibration to increase the quantity and quality of bone in DMD. This intervention is non-invasive, non-pharmacological, involves short treatment sessions, and has been shown to improve bone, decrease fractures, and increase mobility in several populations of disabled children. However, mechanical vibration has not been studied in individuals with muscle disease and before it can be considered as a therapy for DMD, it must irrefutably be determined to be non-damaging to the fragile muscle. The third aim of our project is to work with clinicians and therapists at the University in order to relate our findings on mechanical vibration of mouse bone and muscle to a therapy for individuals with DMD.

Laura P.W. Ranum Ph.D.

(RG) Multisystemic model of RNA toxicity for DM1 and DM2

\$104,500	1/1/2010	12/31/2010	Year 3
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Summary The proposed research will better define the molecular causes and potential reversibility of the multisystemic features of myotonic dystrophy (DM1). Analysis of new mouse models we have developed will allow us to better define the underlying causes of these diseases and the potential to reverse the disease when the transgene is turned off. Understanding the specific molecular changes that occur is important for developing future treatments to stop disease progression.

David D. Thomas Ph.D.

(RG) Interaction of actin with dystrophin and utrophin

\$95,000	7/1/2009	6/30/2010	Year 3
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Summary Researchers will investigate the interactions of dystrophin and utrophin with actin filaments.

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

(DG) Understanding cardiac dystrophin: critical to improving gene therapy for DMD
\$45,000 1/1/2010 12/31/2010 Year 3

Summary This proposal will examine the ability of a truncated dystrophin to replace dystrophin in the heart. Cardiac function will be assessed in several animal models of DMD. These studies will provide critical information for developing the next generation of truncated dystrophins that will improve the treatment of the heart in DMD.

Rochester - Mayo Clinic Rochester

Michael A. Barry Ph.D.

(RG) Cell targeting vectors for muscular dystrophy
\$103,680 7/1/2009 6/30/2010 Year 2
\$102,295 7/1/2010 6/30/2011 Year 3

Summary This project will develop technologies towards the use of "smart" gene therapy vectors that can seek out and target gene delivery to neuromuscular muscle cells in the body for the treatment of Duchenne and other muscular dystrophies.

Andrew George Engel M.D.

(RG) Congenital myasthenic syndromes
\$122,763 1/1/2010 12/31/2010 Year 1
\$122,763 1/1/2011 12/31/2011 Year 2
\$122,763 1/1/2012 12/31/2012 Year 3

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

Bruce Horzodovsky Ph.D.

(RG) Cellular defects associated with ALS2
\$118,750 1/1/2010 12/31/2010 Year 2
\$118,750 1/1/2011 12/31/2011 Year 3

Summary We have been examining a gene that is mutated in a genetic form of ALS, called ALS2. Unlike classical ALS, this juvenile form of the disease appears very early in life (leading to paralysis), but is not quite as severe as the classical form of the disease. The gene mutated in these children codes for a protein called Alsin that plays an important role in preventing neurons from dying. By dissecting Alsin function we will gain new insights into the causes of ALS and in doing so identify new targets for treatment.

St Paul - Gillette Children's Specialty Healthcare

Jason Kelecic DPT

(SG) Moving Forward in the Treatment of Pediatric Neurological Disorders
\$5,000 5/1/2010 5/31/2010 Year 1

Summary The conference, titled "Moving Forward in the Treatment of Pediatric Neurological Disorders," will focus on the latest research and clinical findings for the treatment of neurological disorders for the pediatric population. This course is designed to increase providers' understanding of advances in diagnostic techniques, treatment strategies and research findings for children with pediatric neurological disorders. The faculty features noted clinical experts from across North America. The format will combine large-group plenary sessions with interactive Pearls and Perils sessions and breakout sessions of smaller groups. This format will allow for interchange and communication among professionals from different disciplines who work with patients who have congenital and acquired disorders. This conference is intended for pediatric neurologists, pediatric neurosurgeons, pediatric rehabilitation medicine specialists, pediatric orthopaedic surgeons, primary care physicians, physical and occupational therapists, speech and language pathologists, nurse practitioners, nurses, physician assistants, orthotists and other providers who treat congenital and acquired disabilities. Objectives include: -To identify current scientific advances in the area of pediatric neurological disorders -To translate research into clinical care for people who have cerebral palsy, acquired brain injuries, neuromuscular disorders and other neurological disorders -To examine the clinical benefit

MISSOURI

Columbia - University of Missouri

Dawn D.W. Cornelison Ph.D.

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

\$80,000	7/1/2010	6/30/2011	Year 1
\$80,000	7/1/2011	6/30/2012	Year 2

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

Dongsheng Duan Ph.D.

(RG) Systemic AAV gene therapy in a Duchenne dog model

\$109,250	7/1/2009	6/30/2010	Year 2
\$109,250	7/1/2010	6/30/2011	Year 3

Summary Adeno-associated virus-mediated gene therapy has shown great promise to ameliorate Duchenne muscular dystrophy. In this study we will apply our novel techniques to achieve whole body gene transfer in newborn DMD dogs. The majority of DMD patients can be diagnosed through neonatal screening. Our study will open the door for neonatal gene therapy in human patients in the future.

Christian Lorson Ph.D.

(RG) The role of enhanced muscle in SMA

\$117,475	1/1/2010	12/31/2010	Year 2
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\$120,325	1/1/2011	12/31/2011	Year 3
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Summary Spinal Muscular Atrophy is the leading genetic cause of infantile death, yet there currently is no treatment or cure. This proposal is designed to test a muscle-enhancing compound and a SMN-inducing compound in a mouse model of disease. These results have the potential to identify novel therapeutic avenues for SMA patients.

St. Louis - Saint Louis University

Jindrich Soltys Ph.D., D.V.M.

(RG) Role of complement and its regulatory proteins in EAMG pathogenesis

\$116,740	7/1/2009	6/30/2010	Year 2
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\$118,325	7/1/2010	6/30/2011	Year 3
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Summary Myasthenia gravis (MG) is an autoimmune disease that compromises how well a skeletal muscle responds to transmission from a nerve. The abnormality is caused by the breakdown of the AChR due to complement activation. Complement represent about 30 serum proteins, which interact with the cellular immune response. The ultimate purpose of our investigation is to understand how humoral (complement fixing antibodies) and cellular immunity (T and B cells) respond in the absence of the complement regulatory proteins or when the activity of complement is completely inhibited. Our investigation will provide a rationale for application of complement inhibition as a therapeutic intervention in patients with MG. Complement inhibitors are already used in various disorders, and we believe that similar application in neuromuscular disorders will provide an efficient and alternative way of treatment.

St. Louis - Washington University

Robert Baloh M.D., Ph.D.

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

\$142,407	7/1/2009	6/30/2010	Year 1
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\$133,278	7/1/2010	6/30/2011	Year 2
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\$135,172	7/1/2011	6/30/2012	Year 3
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Summary In ~10% of ALS patients the disease runs in their family. Studying these families has led to the discovery of genes that can cause ALS, and can be used to develop animal models of the disease. This pathway of research led to the development of the SOD1 mouse model, which is a mainstay for testing new therapies for ALS. Our group and others recently found that mutations in a gene called TDP-43 are another important cause of familial ALS. This is exciting because fragments of the TDP-43 protein are found in the motor neurons in most forms of ALS, including the more common form that does not run in families. Therefore, it is likely that understanding how TDP-43 gene mutations cause ALS will provide

completely new insights into how the disease develops. We have continued our work by engineering a mouse which expresses the mutated form of TDP-43 that we discovered in a family with ALS followed at the MDA clinic at Washington University. Our initial evaluation of this mouse shows that it develops a progressive and fatal disease with similarity to ALS. This grant proposal outlines experiments to characterize this mouse model in detail, so that it can effectively be used to develop and test new therapies for ALS.

Anne M. Connolly M.D.

(CRNG) MDA-DMD Center at Washington University

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary We present three clinical proposals that a clinical trials network could rapidly address. First, establish clinical outcome measures in infants, young boys and wheelchair-bound boys and men with DMD. There is a pressing need now to establish outcomes for very young who may be unreliable for testing and for older, weaker boys and men. Second, we propose to establish standard of care for treatment of osteoporosis in boys and men with DMD. A careful prospective trial is needed to determine what and when treatment should be given. Third, we propose to determine if angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are comparable in treatment of cardiomyopathy in DMD. While two trials now show that ACE inhibition delays the onset of cardiomyopathy and prolongs the life of boys with DMD, no human trials of ARBs have been done. This second class of drugs shows beneficial skeletal muscle benefit in mdx mice. Therefore a randomized trial comparing the two would have immediate clinical implications.

Anne M. Connolly M.D.

(RG) Clinical outcome validation in non-ambulatory and young boys/men with DMD

\$250,269	1/1/2010	12/31/2010	Year 1
\$197,688	1/1/2011	12/31/2011	Year 2
\$203,080	1/1/2012	12/31/2012	Year 3

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

Marc I. Diamond M.D.

(RG) Propagation of pathology in neurodegeneration

\$150,000	1/1/2010	12/31/2010	Year 1
\$150,000	1/1/2011	12/31/2011	Year 2

\$150,000 1/1/2012 12/31/2012 Year 3

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

Paul T. Golumbek M.D., Ph.D.

(RG) Prednisolone's therapeutic site of action in mdx mice

\$142,500 7/1/2009 6/30/2010 Year 2

\$142,500 7/1/2010 6/30/2011 Year 3

Summary Prednisolone improves the strength and lifespan of mdx mice and boys with DMD, but we still don't understand how it works. Some believe it works by suppression of the immune system, others think the effect is on muscle directly, while others support a combination of these effects. We have previously investigated the role of three key immune players (complement, T-cells, and B-cells) in order to pinpoint which might worsen muscle strength and which respond to steroid treatment. We showed these players do not worsen the strength of mdx mice and do not account for prednisolone's action. Finding an immune response that worsens strength would allow us to arrest it. By ruling out the immune system we can avoid focusing research on innocent bystanders. Using depletion and genetics approaches, similar to the previous study, we plan to investigate the remaining immune suspects and test for direct muscle effects. Using strength tests as outcome measures (as are used in clinical trials with DMD boys), we will test muscle and immune components, macrophages, granulocytes and Natural killer cells. We will either deplete or disable the immune cells or disable the direct muscle response to steroid in mdx mice, and then assess steroid's clinical actions. Independently, each of the immune cells is capable of attacking sick muscle fibers and each is capable of steroid responsiveness. This work will define steroid's therapeutic mechanism in mdx mouse (and thus DMD) muscle disease.

Didier Hodzic Ph.D.

(RG) Involvement of the LINC complex in Emery-Dreifuss muscular dystrophy

\$118,750 1/1/2010 12/31/2010 Year 3

Summary We hypothesize that mutations of A-type lamins related to EDMD compromise the integrity of the LINC complex, which, in turn, induces a mechanical failure of the whole cell and possibly of muscle tissues. We will test this hypothesis and further examine the *in vivo* consequences of the disruption of the LINC complex. These results could provide a molecular etiology of muscle pathologies linked to A-type lamin mutations.

Jeffrey D. Milbrandt M.D, Ph.D.

(RG) Increased Nmnat activity as treatment for hereditary neuropathies

\$104,500 7/1/2009 6/30/2010 Year 2

\$104,500 7/1/2010 6/30/2011 Year 3

Summary Disease progression in many neuropathies and neurodegenerative conditions like CMT and ALS is correlated with abnormalities in axons, the neuronal extensions that connect neurons to their targets. We have found that increased levels of an enzyme that synthesizes a molecule involved in cellular energy metabolism can protect against axonal degeneration by decreasing the accumulation of naturally occurring toxic substances generated by cellular processes. We will now investigate the cellular pathways that are involved in protecting axons from damage by this enzyme. We will also explore whether altering this enzyme and/or energy pathways can protect against axonal degeneration and slow disease progression caused by mutations associated with hereditary neuropathies.

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

(TRIND) Toxicology and phase I studies of antisense ISIS 333611 in familial ALS

\$239,176 4/1/2008 3/31/2011 Year 2

Summary Some familiar cases of ALS are caused by mutations in superoxide dismutase 1 (SOD1). Mutated SOD1 is likely to benefit patients with SOD1 mutations. ISIS 333611, an antisense oligonucleotides causes degradation of SOD1 RNA and thus protein. We plan to infuse ISIS 333611 into the fluid that surrounds the brain and spinal cord. We are seeking funding for safety testing of this compound first in animals, and then in SOD1 ALS patients.

Alexander Parsadanian Ph.D.

(RG) Therapeutic potential of Neurturin in the G93A mouse model of ALS

\$112,000 7/1/2009 6/30/2010 Year 2

\$112,000 7/1/2010 6/30/2011 Year 3

Summary Neurotrophic factors (NF) have been considered as potential agents for the treatment of motoneuron diseases (MND), including ALS, based on their in vitro and in vivo ability to promote the survival of motoneurons (MN). We focused primarily on members of GDNF Family Ligands (GFLs): GDNF, Neurturin (NTN), Artemin (ART) and Persephin (PSP), which have overlapping but distinct effects on MNs. The in vivo effects of NTN on MNs are not studied in detail. As a proof of concept, we have selected a transgenic approach to address this question. We generated transgenic mice overexpressing NTN in skeletal muscle (Myo-NTN) and in neurons (Thy1-NTN). Based on our preliminary data demonstrating that overexpression of NTN in neurons promotes complete and long-term survival of axotomized MNs, we propose that NTN will have significant beneficial effects in a mouse model of familial ALS. We will study the neuroprotective effects of NTN in G93A-SOD1 mice cross-bred with our transgenic mice overexpressing NTN either in skeletal muscles or in neurons both at anatomical and behavioral levels. We will study the effects of NTN on MN, motor axon, NMJ and terminal Schwann cell degeneration. We will elucidate the mechanisms of NTN action and signaling pathways activated by NTN in vivo. Understanding the mechanisms by which NTN acts on normal and degenerating MNs may give insight into how this NF can be used for treatment of MNDs.

Conrad Weihl M.D., Ph.D.

(RG) ERAD and the unfolded protein response IBMPFD muscle disease

\$104,534 1/1/2010 12/31/2010 Year 2

\$106,131 1/1/2011 12/31/2011 Year 3

Summary Inclusion body myopathies (IBM) are a group of disabling skeletal muscle disorders. Mutations in the protein p97/VCP cause the autosomal dominant multisystem syndrome, IBMPFD, inclusion body myopathy associated with paget's disease of the bone (PDB) and fronto-temporal dementia (FTD). One clear role for p97/VCP is as a facilitator of protein degradation via the ubiquitin-proteasome system (UPS). p97/VCP is essential for the degradation of cytosolic proteasome substrates as well as for endoplasmic reticulum associated degradation (ERAD) of misfolded secreted or transmembrane proteins. It likely performs this role by selectively binding with ubiquitinated substrates via co-factors and transferring them to the 26S proteasome machinery. Currently it is unclear how mutations in p97/VCP cause disease. We plan to explore the role of IBMPFD mutations in p97/VCP on the UPS in skeletal muscle using cultured cells and a transgenic mouse model.

NEW JERSEY

Newark - UMDNJ-New Jersey Medical School

Diego Fraidenraich Ph.D.

(RG) Embryonic stem cells prevent Duchenne muscular dystrophy in mdx mice
\$95,000 1/1/2010 12/31/2010 Year 3

Summary We seek to prevent muscular dystrophy from occurring by supplying wild type embryonic stem cells before the muscle forms. We will inject wild type mouse embryonic stem cells into early mouse embryos predisposed to DMD. Preliminary analyses found that low numbers of embryonic stem cells incorporated into the mouse are sufficient to prevent disease from occurring. We will investigate further the underlying molecular mechanisms whereby the embryonic stem cells exert corrections in skeletal muscle.

Piscataway - UMDNJ--Robert Wood Johnson Medical School

Sarah Ellen Hitchcock-DeGregori Ph.D.

(RG) Tropomyosin in health and disease: bioinformatics and biophysical approaches
\$102,517 1/1/2010 12/31/2010 Year 3

Summary Mutations in tropomyosin cause a number of myopathies, including cardiomyopathies, nemaline myopathies, distal arthrogyryposis, and Cap disease. We will carry out a bioinformatics analysis of the evolution of tropomyosin structure. We will create a phylogenetic tree and the measure the evolutionary rate of each amino acid. This will identify the most conserved residues and lead to new models that will be experimentally tested with the aim of understanding how disease-causing mutations lead to cellular dysfunction and disease.

South Plainfield - PTC Therapeutics, Inc.

Langdon L Miller MD

(MVP) Phase 2a trial of Ataluren in nonambulatory boys with nonsense mutation
DMD/BMD
\$250,000 1/12/2010 4/15/2010 Year 1

Summary Duchenne/Becker muscular dystrophy (DMD/BMD) is a rare genetic disorder that primarily affects boys. Boys with DMD/BMD have muscle weakness

that worsens over time, typically leading to wheelchair dependency, loss of upper extremity function, and death from heart or lung failure in early adulthood. Corticosteroids can slow the progression of the disease, but cause serious side effects and often not used by patients who have lost ambulation. There is no current therapy for the underlying cause of DMD/BMD. Approximately 13% of boys with DMD/BMD have the disease due to a genetic defect called a nonsense mutation. An experimental drug, ataluren (PTC124), has the potential to treat the underlying cause of DMD/BMD in such patients. Preliminary studies of ataluren in mice and humans have yielded encouraging results. An ongoing clinical trial is evaluating the ability of ataluren to improve walking in ambulatory boys with nonsense mutation DMD/BMD (nmDMD/BMD). This project will support an MD

NEW MEXICO

Albuquerque - University of New Mexico

Richard Cripps D. Phil.

(RG) A Drosophila model for mammalian muscular dystrophy

\$113,187	7/1/2010	6/30/2011	Year 1
\$113,187	7/1/2011	6/30/2012	Year 2
\$113,187	7/1/2012	6/30/2013	Year 3

Summary We shall study muscle development in the fruit fly, *Drosophila melanogaster*, to help us understand muscle development and disease in humans. We use *Drosophila* as a model organism because the mechanisms of muscle development in this animal are very similar to those of vertebrates, yet the genetic processes are simpler and more well understood in flies. This project will study the function in *Drosophila* of a gene named *abba*, for which mutations in a related gene in humans have been identified to cause muscular dystrophy. We shall carry out experiments to understand how *abba* works in flies, including characterizing mutants for this gene, which we propose will develop muscular dystrophy in fly larvae. We shall also carry out experiments to define at the molecular level how the protein produced by *abba* functions in muscle, which will tell us a great deal about how the normal gene works in humans. These findings in *Drosophila* will therefore provide insight into normal muscle processes in humans; accordingly, our data will help us to understand how muscle development goes awry in diseased individuals, and will uncover potential mechanisms by which to generate rational therapies for muscle disease.

NEW YORK

Albany - Research Foundation of SUNY - University at Albany

Li Niu Ph.D.

(RG) Making stable aptamers as ALS drug candidates

\$136,872	7/1/2009	6/30/2010	Year 1
\$165,792	7/1/2010	6/30/2011	Year 2
\$102,572	7/1/2011	6/30/2012	Year 3

Summary Excessive activation of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) subtype of ionotropic glutamate receptors has been hypothesized as one of the leading pathogenic mechanisms for amyotrophic lateral sclerosis (ALS). Developing powerful inhibitors to control the excessive receptor

activity is thus a logical therapeutic strategy. In a proof of concept experiment, we have successfully identified a class of AMPA receptor-selective RNA aptamers or RNA inhibitors. The potency of one aptamer rivals any existing AMPA receptor inhibitors ever reported thus far. Unmodified, however, these RNA aptamers are limited in therapeutic applications in vivo by their inherent sensitivity towards ribonucleases, the enzymes that catalyze the degradation of RNA into smaller pieces so that the biological function of the RNA as inhibitors is lost. However, chemical modifications of RNA molecules can turn them into ribonuclease-resistant or biostable aptamers. Thus, making ribonuclease-resistant aptamers is required, as the first step, to translate these aptamers from powerful AMPA receptor antagonists into clinically useful drugs. The specific goal of this proposal is to develop high-affinity, chemically modified aptamers. These chemically modified aptamers will be immediately suitable for testing their neuroprotective effectiveness in ALS cellular and animal models.

Bronx - Albert Einstein College of Medicine of Yeshiva University

Jennifer Troncales Aguilan Ph.D.

(DG) The role of LARGE in the glycosylation of alpha-dystroglycan
 \$45,000 7/1/2009 6/30/2010 Year 2
 \$45,000 7/1/2010 6/30/2011 Year 3

Summary The discovery of the dystrophin gene, which is the cause of Duchenne Muscular Dystrophy, led to the discovery of additional gene mutations that give rise to other muscular dystrophies. These genes encode proteins that form a large complex with dystrophin called the dystrophin glycoprotein complex (DGC). Alpha-dystroglycan (a-DG) is a glycoprotein in the DGC that serves to maintain mechanical stability and function of skeletal muscle. a-DG links the cytoskeleton under the cell membrane to matrix proteins on the cell surface including laminin. Laminin binds to the sugars on a-DG. Abnormalities in the structure or loss of these sugars lead to congenital muscular dystrophies known as dystroglycanopathies. The sugars are transferred to a-DG by enzymes called glycosyltransferases. Six genes thought to encode glycosyltransferases or chaperones that mediate sugar transfer to a-DG have been identified. One of these genes, LARGE, is the basis of MDC1D. Overexpression of LARGE bypasses defects in several dystroglycanopathies by restoring a-DG function. However, the biochemical reactions catalysed by LARGE are unknown. The aim of this proposal is to determine how LARGE modifies a-DG in order to understand the biochemical basis of MDC1D and to determine how LARGE may be used in the treatment of dystroglycanopathies.

Chi-Wing Chow Ph.D.

(RG) Exosomal trafficking and CMT1C disease
 \$85,500 1/1/2010 12/31/2010 Year 2
 \$85,500 1/1/2011 12/31/2011 Year 3

Summary Mutations in protein SIMPLE account for the etiology in CMT1C patients. Duplication of PMP22 in CMT1A patients exhibit similar electrophysiological parameters and histopathological observations as in CMT1C, suggesting molecular interaction between SIMPLE and PMP22. The molecular basis of SIMPLE, however, is not known. My laboratory recently discovered that SIMPLE participates in vesicular trafficking to EXTRACELLULAR space. Indeed, secreted SIMPLE is found in exosomes, unique extracellular fractions that contain 50-90 nm microvesicles. Major function of the exosome is to facilitate cell-cell autocrine/ paracrine

communications and genetic exchanges by delivering/ disposing endosomal cargos to extracellular milieu. Our recent data further demonstrated that missense mutations found in CMT1C patients abolished the ability of SIMPLE to localize to the extracellular exosomes. Expression of PMP22, mimicking duplication of PMP22 in CMT1A, also reduced exosomal secretion of SIMPLE, supporting genetic interaction of CMT1C and CMT1A. Here, we propose that Schwann cells containing CMT1C SIMPLE mutants cause defects in myelination, in part, due to the lack of exosome-mediated communication.

Amber Wells Ph.D.

(DG) Investigating the effects of mRNA targeting and translation on muscle adhesion

\$45,000 1/1/2010 12/31/2010 Year 3

Summary We propose to investigate how CMA (cell-matrix attachments) mRNAs are localized to CMAs and how the localized synthesis of protein affects CMA adhesion.

Cold Spring Harbor - Cold Spring Harbor Laboratory

Adrian R. Krainer Ph.D.

(RG) Correction of the SMN2 splicing defect in SMA mice using antisense oligonucleotides

\$241,300 7/1/2009 6/30/2010 Year 3

Summary Researchers will use antisense oligos to SMN2 for correct splicing to form SMN1 the form missing in SMA.

New York - Columbia University College of Physicians and Surgeons

Howard J. Worman M.D.

(RG) Treatment of cardiomyopathy in Emery-Dreifuss muscular dystrophy

\$103,631 7/1/2010 6/30/2011 Year 1

\$103,631 7/1/2011 6/30/2012 Year 2

\$103,631 7/1/2012 6/30/2013 Year 3

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

New York - Columbia University Medical Center

Veronica Hinton Ph.D.

(RG) Development of language skills among boys with Duchenne muscular dystrophy

\$96,748	7/1/2009	6/30/2010	Year 2
\$99,003	7/1/2010	6/30/2011	Year 3

Summary The objective of this study is to examine development of language skills in boys with Duchenne muscular dystrophy (DMD). Children with DMD have been shown to be at risk for having delayed language skills, and many have a limited ability to listen to and hold verbal information in immediate memory. For the child with DMD, little attention has been given to the real-life issues associated with cognitive deficits. Most clinical attention is aimed at treating the physical aspects of the disease and slowing the progressive muscular weakness. Children with DMD may struggle at learning academics, and have poor social skills. Although these concerns are not life-threatening, they may compromise a child's optimal enjoyment of his life, yet can be mediated and improved, especially if identified early. This research aims to clarify the nature of the development of these issues. The study will build on our ongoing work that has already identified language skills in two groups of children with DMD. Younger boys who have been followed over two years will be followed longer to examine the acquisition of academic skills. Older school-aged boys who have had detailed assessment of their language skills will be tested on academic tests and questioned about their social skills and quality of life. This study offers an exceptional opportunity to investigate ways to ameliorate an area of potentially considerable stress in children with DMD.

Michio Hirano M.D.

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

\$111,870	1/1/2010	12/31/2010	Year 2
\$111,905	1/1/2011	12/31/2011	Year 3

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

Oliver Hobert Ph.D.

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

\$124,837	7/1/2010	6/30/2011	Year 1
\$124,837	7/1/2011	6/30/2012	Year 2
\$124,837	7/1/2012	6/30/2013	Year 3

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

Hiroshi Mitsumoto M.D.

(CRNG) MDA Clinical Research Network

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary The Eleanor and Lou Gehrig MDA/ALS Research Center at Columbia University has been a leader in providing state-of-the-art multidisciplinary care and management for patients with ALS and their families. We propose to develop a scale for clinically meaningful changes. The Network will be instrumental in developing a research biobank and effective educational activities for patients with ALS and their families.

Hiroshi Mitsumoto M.D.

(RRG) WOWS ALS research at the Eleanor & Lou Gehrig MDA/ALS Research Center at CUMC

\$402,512 8/1/2008 12/31/2012 Year 1

Summary The MDA Wings Over Wall Street (WOWS) fund has enormously helped initiate and maintain a high output of clinical and translational research activity in ALS at the Eleanor and Lou Gehrig MDA/ALS Research Center, Columbia University since 2002. In fact, this fund has turned our ALS Center into one of the most prominent ALS Centers in the Nation and led to the subsequent establishment of the Center for Motor Neuron Biology and Disease at Columbia University. We had a number of breakthroughs and important contributions in the ALS clinical and basic science fields. With continued support from the MDA Wings, we will continue clinical and translational research projects to make every effort to find the cause and cure of ALS.

Hiroshi Mitsumoto M.D.

(RG) Clinically meaningful changes (CMC) scale in the MDA/ALS Clinical Network

\$139,015 7/1/2009 6/30/2010 Year 1
\$121,607 7/1/2010 6/30/2011 Year 2
\$140,160 7/1/2011 6/30/2012 Year 3

Summary In this project, we will develop a Clinically Meaningful Changes (CMC) Scale in patients and caregivers. It will assess the overall judgment as to how much medication or other treatments improve the disease in patients' and caregiver's perception. Using the newly approved 5 MDA Clinical Research Network Centers, we will study a total of 100 pairs of patients and caregivers. In Aim 1, we will study the reliability of new scales, including a) a scale asking overall impression of the changes, b) a scale for a subject attaining a goal they realistically want to see, c) retrospective analysis of health states based on patient report, and d) "trade-offs" analyses of treatment benefits versus side effects. We will analyze how reliable they are if we interview by person or by telephone. We will confirm that the two interview methods would be equally reliable. In Aim 2, we will measure how many changes occur in these scales over 6 months. We will compare changes in these scales and ALSFRS-R, the standard scale for ALS disease progression. Using a statistical analysis, we will find the best combination of scales which we would call the final CMC scale. In Aim 3, we will test the usefulness of the CMC scale in a real clinical trial. In Aim 4, we will also study the clinical meaning of the ALSFRS-R in relation to the level of disability and use of medical treatment and also study how much impact a unit change in ALSFRS-R causes in patients with ALS.

Hiroshi Mitsumoto M.D.

(RRG) 2008 Wings Over Wall Street Projects at the Columbia MDA/ALS Center

\$176,583 7/1/2009 6/30/2011 Year 1

Summary With continued support from the MDA Wings Over Wall Street (WOWS), our research projects in patient-oriented research have been diverse and

remain very active. There will be 4 projects. Project 1 is "Prospective study of oxidative stress in patient ALS." The original project has been funded by the MDA Research grant and it is well on its course and has recruited more than 50 patients. This project is intended to be a national multicenter study and the NIH grant we submitted for these goals has received a high priority score of 121 (8.1 percentile) which makes funding for this NIH grant almost certain. Thus, as we hoped, the oxidative stress project will be a prospective 11 multicenter study. However, across the board, an automatic 15% reduction to the proposed budget will take place at NIH. Therefore, the WOWS fund will be needed to support the offset as much as possible to undertake a large multicenter study. We will investigate if increased oxidative stress (based on biomarkers and markers identified by environmental, lifestyle, and psychological factors) is associated with poor prognosis of ALS. We also try to find if subsets of ALS are associated with oxidative stress. Furthermore, oxidative stress biomarkers and markers detected by environmental, lifestyle, and psychological factors are closely corresponding. Project 2 is to investigate the underlying mechanisms of dyslipidemia.

Hiroshi Mitsumoto M.D.

(RG) Ambispective study of oxidative stress in the etiology and progression of ALS
 \$95,000 7/1/2009 6/30/2010 Year 3

Summary ALS patients will be tested for oxidative injury and will determine if continued exposure to oxidative stressors is detrimental in ALS.

Umrao R. Monani Ph.D.

(RG) Investigating the temporal requirements of the SMN protein in SMA
 \$112,370 1/1/2010 12/31/2010 Year 1
 \$112,370 1/1/2011 12/31/2011 Year 2
 \$112,370 1/1/2012 12/31/2012 Year 3

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

Livio Pellizzoni Ph.D.

(RG) Regulation of SMN function in ribonucleoprotein assembly
 \$127,520 7/1/2009 6/30/2010 Year 1
 \$116,283 7/1/2010 6/30/2011 Year 2
 \$116,085 7/1/2011 6/30/2012 Year 3

Summary Spinal Muscular Atrophy (SMA) patients have homozygous deletions or mutations in the survival motor neuron (SMN1) gene and retain at least one copy of the nearly identical SMN2 gene. The SMN2 gene produces reduced amounts of functional SMN that cannot fully compensate for the absence of SMN1, leading to specific degeneration of motor neurons in the spinal cord and atrophy of skeletal muscle. Currently, most therapeutic strategies for SMA aim at increasing the expression of functional SMN from the SMN2 gene. The SMN protein has a well-established function in RNA metabolism that is regulated in a tissue-specific and time-dependent manner by unknown cellular factors. Our hypothesis is that targeting these factors will enhance the low levels of SMN activity in cells of SMA

patients and represent an alternative therapeutic approach. The project will explore this possibility by studying the cellular factors that control SMN activity and their mechanism of action in motor neurons and skeletal muscle cells. The effect of modulating the activity of these regulatory factors in cellular and animal models of SMA will also be analyzed. Beyond providing fundamental insights into the biology of SMN, our studies will identify potential targets for the development of novel therapeutic approaches for SMA.

Patricia Richard Ph.D.

(DG) Function of the putative helicase senataxin in ALS4.

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary One form of ALS, juvenile amyotrophic lateral sclerosis or ALS4 is a neurological disorder caused by mutations in the Senataxin (SETX) gene. I am interested in understanding how mutations in this yet uncharacterized gene can lead to this disease. By using biochemical and molecular biology approaches, I will investigate the function of SETX in normal cells and in cells expressing mutants of SETX.

Eric A. Schon Ph.D.

(RG) Pharmacological approaches to treat human mitochondrial diseases

\$130,146	7/1/2009	6/30/2010	Year 1
\$133,291	7/1/2010	6/30/2011	Year 2
\$136,506	7/1/2011	6/30/2012	Year 3

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

Ji-Yeon Shin Ph.D.

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

\$60,000	7/1/2010	6/30/2011	Year 1
\$60,000	7/1/2011	6/30/2012	Year 2
\$60,000	7/1/2012	6/30/2013	Year 3

Summary Although diagnosis for Emery-Dreifuss muscular dystrophy (EDMD) has been improved by the discovery of the most common genetic mutations that cause

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

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

Dale Lange M.D.

(RG) Safety and efficacy of SOD1 inhibition by pyrimethamine in familial ALS

\$200,000	4/1/2009	3/31/2010	Year 1
\$190,000	4/1/2010	3/31/2011	Year 2
\$95,000	4/1/2011	3/30/2012	Year 3

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

Giovanni Manfredi M.D., Ph.D.

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

\$99,846	7/1/2009	6/30/2010	Year 1
\$99,846	7/1/2010	6/30/2011	Year 2
\$99,846	7/1/2011	6/30/2012	Year 3

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

animal models of OXPHOS defects. Our goal is to identify new avenues for treatment of mitochondrial disorders.

New York - Memorial Sloan-Kettering Cancer Center

Mary Baylies Ph.D.

(RG) Investigation of mechanisms underlying myonuclear positioning

\$102,195 1/1/2010 12/31/2010 Year 2

\$105,021 1/1/2011 12/31/2011 Year 3

Summary Critical to the performance of muscle is its correct formation, maturation and interaction with both the nervous system and tendons. During normal development, myonuclei localize in the center of myofibers and subsequently migrate to the fiber surface, becoming located beneath the sarcolemma and distributed evenly along the length of myofiber. Many myopathies and dystrophies are characterized by a redistribution of these myonuclei from the external positions to central positions within a myofiber. Despite the importance of nuclear positioning for proper muscle function and clinical diagnosis, very little is known about this process. To address this gap in knowledge, we conducted a forward genetic screen in the model organism *Drosophila melanogaster* to find genes responsible for nuclear positioning. Our screen has revealed a class of mutants that show defects in nuclear positioning. We have investigated one member of this class and have mapped this mutation to a gene that we named *swoosh*. Live imaging analysis indicates that, in contrast to wildtype muscle nuclei, *swoosh* mutant nuclei fail to migrate and position. In this project we will investigate the *swoosh* protein and its role in correctly positioning myonuclei. Based on our preliminary data, we hypothesize that *swoosh* functions to correctly move nuclei along microtubules to their final position, and that it accomplishes this by serving as an adaptor between the microtubule motor kinesin and the nuclear protein lamin.

New York - New York University School of Medicine

James Salzer M.D., Ph.D.

(RG) Characterization of signaling pathways in Charcot-Marie-Tooth disease

\$106,871 1/1/2010 12/31/2010 Year 2

\$110,166 1/1/2011 12/31/2011 Year 3

Summary Inherited neuropathies (CMT) are a major source of disability and pain that result in part from loss of myelin (dysmyelination) around nerve fibers. While there have been significant advances in determining the genetic basis of inherited neuropathies, progress in understanding how these genetic mutations cause neuropathy and how to treat them, has remained limited. Our laboratory is characterizing signals that control whether cells make and maintain myelin around nerves. Some of these signals appear to be abnormally activated in these neuropathies and may therefore be candidates for therapeutic intervention. We have identified neuregulin/erbB signaling and a downstream target, mTOR (the mammalian target of rapamycin), as being aberrantly activated in nerve injury and in a neuropathy model. mTOR signaling enables over growth of a variety of cells and is therefore being targeted in cancer clinical trials. Our preliminary studies suggest that inhibiting mTOR activity effectively blocks Schwann cell proliferation and demyelination that is triggered by aberrant growth factors. We propose to build on these initial studies by: i) further characterizing these signaling pathways in

rodent models of CMT ii) developing tissue culture models of CMT that will allow us to study these signaling pathways in more detail, and iii) determining whether we can prevent dysmyelination in CMT models by blocking mTOR and other aberrant signals with pharmacological inhibitors.

Rochester - University of Rochester

Robert Griggs M.D.

(RG) Recruitment for HYP HOP phase III Trial in the periodic paralyses

\$53,505	7/1/2009	6/30/2010	Year 2
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\$54,013	7/1/2010	6/30/2011	Year 3
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Summary A clinical trial called HYP HOP is being conducted to see whether either of two drugs, acetazolamide, or dichlorphenamide, helps to decrease attacks of weakness in hyper-periodic paralysis and hypo-periodic paralysis and whether either one helps to prevent the permanent weakness that develops in these diseases. This can provide physicians with a standard treatment for the diseases. NIH has funded the study but additional funds are requested to help to complete the study.

Robert Griggs M.D.

(SG) Treatment Strategies for Neuromuscular Diseases: The Challenge of Recruitment

\$20,000	8/1/2010	12/31/2010	Year 1
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Summary This request proposes an international conference: "Treatment Strategies for Neuromuscular Diseases: The Challenge of Recruitment", developed by a committee of translational scientists and leaders of the Muscle Study Group (MSG). The forum will bring together expert translational and clinical scientists from academia, NIH, foundations, and pharma with trainee investigators in a collegial, informal, isolated environment. It is open to attendance from MSG, other groups, and individuals with no study group affiliation. It will cover topics from bench-to-bedside to: discuss effective recruitment strategies for volunteers with rare neuromuscular diseases; look at effective methods of recruiting and mentoring young neurologists starting out in neuromuscular research; provide training for junior faculty for developing novel treatment strategies for neuromuscular disease; offer state-of-the-art presentations with a translational focus by laboratory and clinical investigators whose work is being brought into clinical trial; afford interaction/development of collaboration among established investigators and between senior and new investigators. It is expected that the seminar may lead to new collaborations. The conference's scientific emphases will be on fundamental and experimental therapeutic aspects of inflammatory muscle diseases and strategies for bringing gene therapy into clinical trials. Attendees will be invited to participate in an interactive poster discussion session.

Chad R. Heatwole M.D.

(TRIG) The development and use of disease-specific instruments for muscular dystrophies

\$67,810	4/1/2009	3/29/2010	Year 2
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\$76,336	4/1/2010	3/30/2011	Year 3
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Summary This study is in response to a national call for clinically meaningful outcome measures in muscular dystrophy research. This project will develop, test, and validate a myotonic dystrophy disease-specific instrument of quality-of-life for

use in clinical trials. The infrastructure used to create this instrument will be utilized to develop additional disease-specific instruments for both facioscapulohumeral muscular dystrophy and myotonic dystrophy type-2. The project will produce three viable research instruments, create the necessary infrastructure for further quality-of-life instrument development, and promote meaningful outcome measures in muscular dystrophy research.

Masayuki Nakamori M.D., Ph.D.

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

\$60,000	7/1/2009	6/30/2010	Year 1
\$60,000	7/1/2010	6/30/2011	Year 2
\$60,000	7/1/2011	6/30/2012	Year 3

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

Paul Twydell D.O.

(RG) Therapeutic trial of potassium and acetazolamide in Andersen-Tawil syndrome

\$33,534	5/1/2009	4/30/2010	Year 2
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Summary This study will attempt to determine if the medications potassium and acetazolamide will help patients with the form of periodic paralysis called Andersen-Tawil Syndrome (ATS). The study is being carried out to see if these drugs help the muscle and/or heart function in ATS patients. It will also help researchers learn how to perform studies in this rare disease in which only a few patients are available to participate.

NORTH CAROLINA

Chapel Hill - The University of North Carolina at Chapel Hill

Joe Kornegay D.V.M., Ph.D.

(TRIG) Natural history and immunological parameters in the GSHPMD dog

\$330,432	1/1/2010	12/31/2010	Year 2
\$269,578	1/1/2011	12/31/2011	Year 3

Summary Before initiating gene therapy in Duchenne muscular dystrophy, the nature of the immune response to dystrophin protein or viral vector capsid antigens must be determined. Dogs with golden retriever muscular dystrophy (GRMD) express some dystrophin and should, therefore, be partially tolerized. In contrast, German shorthaired pointers with muscular dystrophy (GSHPMD) have a large deletion that encompasses the entire dystrophin gene. The complete absence of dystrophin provides a "clean" background in which to dissect the relative contributions that dystrophin and viral antigens make to the immune response. In this study, we will first systematically study the natural history of both GRMD and GSHPMD dogs from 3 months to 1 year of age using functional tests, pathology, and MRI. We will then conduct a series of experiments in both GRMD and GSHPMD

dogs to determine the nature of their immune response to viral-mediated dystrophin gene therapy.

Charlotte - Carolinas Medical Center

Qi Lu Ph.D.

(SG) Second International Workshop for Glycosylation Defects in Muscular Dystrophies
\$7,500 11/1/2010 11/30/2010 Year 1

Summary Since the first workshop in May 2008, progress has been made in the research areas of muscular dystrophies associated with defects in glycosylation of alpha-dystroglycan. Most significantly, new data from Dr Campbell's laboratory demonstrates that O-mannosyl phosphorylation is required for the functions of alpha-DG; viable animal models with point mutations in the FKRP gene have become available in our lab. However, our understanding of the functional glycosylation of the alpha-DG and the pathways from mutations of the known genes to muscular dystrophy is still limited. No effective therapy has been identified. This workshop will focus on the possible pathways involving the functional glycosylation of the alpha-DG; the mechanisms in the development of dystroglycanopathies, and the potentials of developing novel experimental therapy to the diseases.

Susan Sparks M.D., Ph.D.

(RRG) Improved diagnosis and clinical assessment of limb-girdle muscular dystrophy
\$112,712 7/1/2010 6/30/2011 Year 1

Summary Limb-girdle muscular dystrophy (LGMD) is largely a descriptive term for a molecularly heterogeneous group of muscular dystrophies with onset in childhood or adulthood that is characterized by proximal muscle weakness. LGMD are classified into two groups based on the mode of inheritance, type 1 for autosomal dominant and type 2 for autosomal recessive. Each type is further subdivided depending on the molecular etiology, designated by a letter in the order they were discovered (i.e. LGMD1A-E and LGMD2A-N). Identification of new genes involved in the pathogenesis of LGMD has occurred rapidly, however, due to the rarity of some of the subtypes it has been a challenge. Molecular clarification has resulted in the elucidation of common pathways of pathogenesis, as well as important differences between subtypes of LGMD. However, the diagnosis of an individual with LGMD is still laborious and often incomplete. The community has identified lack of natural history studies as a major gap in our knowledge base and a significant barrier to the development of effective clinical trials in LGMD. In addition, the lack of validated clinical trial endpoints makes it near impossible to transition potential therapeutics through rigorous clinical trials into routine treatments for LGMD. This proposal aims to improve the genetic diagnosis of LGMD, comprehensively evaluate individuals with genetically identified LGMD, and follow potential outcome measures longitudinally in patients with LGMD.

Durham - Duke University Medical Center

Paul Rosenberg M.D.

(RG) The role of STIM1 in muscle disease and performance
\$135,911 1/1/2010 12/31/2010 Year 1
\$135,911 1/1/2011 12/31/2011 Year 2

\$135,911 1/1/2012 12/31/2012 Year 3

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

Jonathan Stiber M.D.

(RG) The role of Homer proteins in Duchenne's muscular dystrophy

\$110,000 7/1/2009 6/30/2010 Year 1

\$110,000 7/1/2010 6/30/2011 Year 2

Summary Transient receptor potential (TRP) channels are a family of ion channels, several of which are expressed in muscle and activated by stretch. Recently, abnormal TRP channel activity has been shown to play a role in the muscle damage seen in Duchenne's muscular dystrophy. Because the scaffolding protein Homer 1 has been shown to regulate TRP channels, we hypothesized that Homer proteins play a significant role in muscle function. Mice lacking Homer 1 exhibit a myopathy characterized by muscle weakness and abnormal TRP channel activity. In addition, we observed a deficiency in Homer 1 protein levels in mouse models of muscular dystrophy. Understanding the role of Homer proteins in the disease progression of muscular dystrophy will provide important insights into the development of multiple strategies aimed at combating this lethal disorder including those targeting stretch-activated channel blockade and growth factor stimulation.

Winston-Salem - Wake Forest University Health Sciences

Oswaldo Delbono M.D., Ph.D.

(RG) Calcium signaling in muscular dystrophy

\$95,000 7/1/2009 6/30/2010 Year 2

\$95,000 7/1/2010 6/30/2011 Year 3

Summary The long term goal of this project is to elucidate the underlying mechanisms and design a successful therapy for Duchenne Muscular Dystrophy (DMD). In this project, we will test the hypothesis that decreased intracellular calcium availability in response to cell stimulation results in skeletal muscle weakness beyond the period of active tissue damage. We will also test the premise that improving cell excitability-Ca²⁺ availability coupling will lead to increased muscle strength.

OHIO

Cincinnati - University of Cincinnati

Rhett A. Kovall Ph.D.

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

\$96,755 7/1/2009 6/30/2010 Year 1

\$93,939	7/1/2010	6/30/2011	Year 2
\$96,147	7/1/2011	6/30/2012	Year 3

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

Tom Thompson Ph.D.

(RG) Towards the development of alternative anti-myostatin therapeutics

\$94,050	7/1/2009	6/30/2010	Year 2
\$94,050	7/1/2010	6/30/2011	Year 3

Summary Myostatin is a naturally occurring protein that inhibits muscle growth. Strong evidence supports the therapeutic potential of myostatin inhibitors for muscular dystrophy. On the other hand, several naturally occurring proteins exist that bind and neutralize myostatin. These includes the antagonist follistatin (FS), which forms a nearly unbreakable complex with myostatin. Our research will focus on characterizing the structure of myostatin with the antagonist FS, and will develop novel strategies to inactivate myostatin with FS. These efforts will facilitate design of novel anti-myostatin molecules with potential to improve muscle growth.

Cleveland - Cleveland Clinic Foundation

Andrea N. Ladd Ph.D.

(RG) Alternative splicing programs in normal muscle and myotonic dystrophy

\$104,500	7/1/2009	6/30/2010	Year 2
\$104,500	7/1/2010	6/30/2011	Year 3

Summary Myotonic dystrophy (DM) is an inherited disease that affects 1 in 8500 individuals worldwide. Symptoms of the disease include myotonia (i.e., the inability to relax voluntarily contracted muscles), muscle wasting, cardiac conduction defects, insulin resistance, cataracts, testicular atrophy, and cognitive dysfunction. In families afflicted with this disease, children may also be born with a severe congenital form of DM characterized by deficiencies in muscle development and mental retardation. An important connection has been made between the development of disease in DM patients and a molecular process called alternative splicing. Alternative splicing allows the production of multiple proteins with different functions from a single gene. Alternative splicing can determine the fate of cells: what kind of cells form, how they function, and even whether they live or die. We previously identified a family of related proteins that regulate alternative splicing in muscle and are disrupted in DM. Inappropriate alternative splicing of genes regulated by these proteins has been shown to contribute to symptoms of the disease, including myotonia, the hallmark of DM. The goals of my research are to investigate the role of these proteins in normal muscle development, and to test

whether repressing the function of these proteins may be used to reverse the molecular defects in alternative splicing that contribute to DM, thus alleviating the disease.

Lan Zhou M.D., Ph.D.

(RG) Therapeutic effects of imatinib on Duchenne muscular dystrophy

\$88,344	7/1/2009	6/30/2010	Year 2
\$83,391	7/1/2010	6/30/2011	Year 3

Summary Imatinib is an FDA-approved drug with promising anti-inflammatory and anti-fibrotic therapeutic applications. Researchers will test whether imatinib can reduce muscle inflammation and fibrosis in DMD to improve muscle function and survival using the DMD mouse models, mdx and mdx/utrn+/-.

Columbus - Ohio State University

Arthur H.M. Burghes Ph.D.

(RG) How do SMN missense mutations affect RNP assembly and how does this cause SMA?

\$107,321	7/1/2009	6/30/2010	Year 1
\$104,461	7/1/2010	6/30/2011	Year 2
\$103,892	7/1/2011	6/30/2012	Year 3

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

Patrice Hamel Ph.D.

(RG) Unraveling the mitochondrial redox pathway in cytochrome c maturation

\$113,249	1/1/2009	3/31/2010	Year 3
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Summary Two c-type cytochrome assembly factors will be studied to determine how cytochrome c is assembled and other factors that may be involved in the process of energy production and cellular death.

Velimir Matkovic M.D., Ph.D.

(RG) Skeletal development in boys with Duchenne muscular dystrophy (DMD)

\$127,610	1/1/2009	3/31/2010	Year 3
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Summary Studies of bone mass in DMD patients will determine from where the loss of bone mass may occur. In addition the use of steroids will be evaluated relative to bone mass.

Jennifer M. Peterson Ph.D.

(DG) NF-kappaB signaling in dystrophic cardiomyopathy

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

Summary The long-term goal of this project is to determine the role of a signaling pathway called nuclear factor-kappaB (NF-kB) in the progression of heart disease that occurs in Duchenne (DMD) and Becker (BMD) muscular dystrophies. Our previous work has demonstrated that NF-kB signaling plays an important role in skeletal muscle disease progression, however the implications of NF-kB signaling in dystrophic heart muscle remain unknown. This study will provide significant insight into the role of the NF-kB signaling pathway in dystrophy-associated heart disease and determine if this signaling pathway has the potential to be targeted for the treatment of DMD and BMD.

Jill Rafael-Fortney Ph.D.

(RG) Investigation of a new treatment target for heart failure in muscular dystrophy

\$126,923	1/1/2010	12/31/2010	Year 1
\$126,923	1/1/2011	12/31/2011	Year 2
\$126,923	1/1/2012	12/31/2012	Year 3

Summary At least 95% of Duchenne muscular dystrophy (DMD) patients develop cardiomyopathy. As therapies to protect respiratory function improve, DMD patients live longer, and the chance of heart failure will approach 100%. Supporting this prediction, Becker muscular dystrophy patients with milder skeletal muscle disease all develop severe cardiac disease. We have discovered that the claudin-5 protein is deficient specifically in heart muscle cells in a muscular dystrophy mouse model that exhibits heart failure. Claudin-5 reductions occur in a time-frame that makes it an excellent candidate as a therapeutic target. We have also identified specific reductions of claudin-5 in at least 60% of patients with heart failure, demonstrating the clinical relevance of this protein and further supporting that claudin-5 may be a key "switch" from many forms of cardiomyopathy to progression of heart failure. Claudin-5 therefore represents a novel potential therapeutic target for treatment of DMD related cardiomyopathy and heart failure. In this study, we will define the mechanisms specific to claudin-5 deficiency, determine claudin-5 levels in other forms of muscular dystrophy, and determine whether exogenous claudin-5 expression is sufficient to prevent heart failure in muscular dystrophy mouse models. This study will directly address the potential of a novel protein as a treatment target for prevention of heart failure in muscular dystrophy patients.

Columbus - Research Institute at Nationwide Children's Hospital

Scott Q. Harper Ph.D.

(DG) RNAi therapy for facioscapulohumeral muscular dystrophy

\$45,000	7/1/2009	6/30/2010	Year 3
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Summary Researchers will test if RNAi against FRG1 will improve symptoms in a mouse model of FSHD.

Jerry Mendell M.D.

(RG) Treatment of the dystrophin deficient cardiomyopathy

\$315,176	7/1/2009	6/30/2010	Year 1
\$306,805	7/1/2010	6/30/2011	Year 2
\$309,841	7/1/2011	6/30/2012	Year 3

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

Jerry Mendell M.D.

(RRG) Vascular delivery gene therapy trial for LGMD2D

\$22,168	10/1/2009	9/30/2010	Year 1
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Summary Currently there is very limited treatment for muscular dystrophy. Supportive and respiratory care have helped prolong life but quality of life is very limited. We have found a way to restore the missing gene in limb-girdle muscular dystrophy using a virus to deliver the defective gene through the circulation. In this study we will replace the defective gene to the upper limb muscles of the legs in patients with LGMD2D, alpha-sarcoglycan deficiency. The objective will be to prolong ambulation.

Jerry Mendell M.D.

(MVP) Improve limb strength by vascular delivery of the alpha-sarcoglycan gene in LGMD2D

\$420,374	2/10/2010	12/31/2010	Year 1
\$38,440	1/1/2011	12/31/2011	Year 2

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

Jerry Mendell M.D.

(CRNG) Request to be a designated MDA DMD Clinical Research Center

\$100,000	8/1/2009	7/31/2010	Year 2
\$100,000	8/1/2010	7/31/2011	Year 3

Summary The overall premise and comprehensive theme for the MDA DMD Clinical Research Center (CRC) at Nationwide Children's Hospital (NCH) is that the heart of Duchenne muscular dystrophy patient is a critical, yet understudied, component of this devastating disease. While we are all encouraged by the progress of potential treatments evolving for skeletal muscle, we have a limited repertoire of treatments for cardiac muscle. In order to rescue the cardiac dystrophinopathy we must: 1) know more about the natural history of the dilated cardiomyopathy and associated cardiac arrhythmias; 2) establish the benefit of current treatments; and 3) determine if additional approaches are necessary to sustain efficacy.

Christopher Pierson M.D., Ph.D.

(RG) Follistatin gene therapy in mouse models of myotubular myopathy

\$85,613	1/1/2010	12/31/2010	Year 1
\$85,613	1/1/2011	12/31/2011	Year 2
\$85,613	1/1/2012	12/31/2012	Year 3

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

OREGON

Eugene - University of Oregon

Andrew Berglund Ph.D.

(RG) A small molecule approach to myotonic dystrophy

\$121,802	7/1/2009	6/30/2010	Year 2
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Summary Myotonic dystrophy is caused by an expansion of CTG repeats at the genomic level. These CTG repeats are made into RNA (CUG repeats) and become toxic molecules within the cell. The CUG repeat RNA molecules are toxic because they sequester a protein factor, disrupting its function as well as increasing the cellular levels of other protein factors involved in myotonic dystrophy. The goal of this project is to determine the ability of a small molecule (and related molecules) that binds the toxic CUG repeats to reverse the molecular defects associated with myotonic dystrophy. This small molecule or a derivative of it could lead to a therapy for patients with myotonic dystrophy.

PENNSYLVANIA

Philadelphia - The Children's Hospital of Philadelphia

Carsten Bonnemann M.D.

(RG) Allele-specific RNAi mediated knockdown of dominant negative Col6 mutations

\$118,750	7/1/2009	6/30/2010	Year 2
\$118,750	7/1/2010	6/30/2011	Year 3

Summary Mutations in the three genes coding for collagen type VI cause the severe congenital muscular dystrophy type Ullrich, as well as the milder type Bethlem. Mutations in Bethlem are always dominant, whereas mutations in the more severe Ullrich type were thought to be recessive, but more recent data suggests that dominant mutations underlie at least 50% of cases of Ullrich, making dominant mutations the predominant mutation mechanism in collagen VI. The potential severity of these mutations results from their ability to act in a dominant negative fashion, i.e., the mutant gene product is able to negatively interfere with any normal collagen VI formed. This study is concerned with developing strategies to selectively knock down the mutant gene transcript using RNA interference technology. We will be developing oligo- as well as viral-based systems to deliver RNA interference to cells and to experimental animals with the goal of improving collagen VI production by interfering with the mutant gene product, ultimately ameliorating the disease.

David Lynch M.D., Ph.D.

(TRIG) Clinical Research Network for Friedreich's Ataxia

\$140,878	3/1/2009	5/31/2010	Year 3
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Summary Using recent support from the MDA and FARA, a group of investigators collaborated on development of clinical measures that can quantitatively assess FA. While a large amount of measure refinement remains to be performed, the data from their collaboration provide a framework for further investigation and for creating a network for performing further clinical translational research including clinical trials.

David Lynch M.D., Ph.D.

(TRIG) Clinical Research Network for Friedreich's Ataxia

\$332,613	6/1/2010	5/31/2011	Year 1
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Summary This study will create research infrastructure for clinical research in Friedreich's Ataxia by supporting background work of a group of core investigators. These investigators will quantitatively assess clinical features of this disorder and match clinical data with biological specimens. The data will be efficiently tabulated through a data coordinating center. This will allow many individuals to investigate mechanisms of clinical change in Friedreich's ataxia using the data accumulated.

Philadelphia - University of Pennsylvania

Stephen Baylor M.D.

(RG) Comparison of calcium signaling in muscle fibers of normal and mdx mice

\$109,325	1/1/2010	12/31/2010	Year 3
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Summary This project will compare intracellular calcium levels in mdx mice (an animal model of DMD) and normal mice to quantify the ways in which intracellular calcium signaling may be altered in DMD. The knowledge gained should lead to an

increased understanding of the patho-physiological changes that occur in DMD and, possibly, to identification of new therapeutic targets for treatment of this disease.

Nancy M. Bonini Ph.D.

(RG) Novel pathway and compound modifiers for myotonic dystrophy

\$87,020	7/1/2009	6/30/2010	Year 1
\$75,534	7/1/2010	6/30/2011	Year 2
\$77,774	7/1/2011	6/30/2012	Year 3

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

Clara Franzini-Armstrong Ph.D.

(RG) IP3 receptors in myonuclei of normal and diseased muscle models

\$73,025	7/1/2009	6/30/2010	Year 2
\$75,242	7/1/2010	6/30/2011	Year 3

Summary Calcium is a ubiquitous intracellular messenger, responsible for initiating events, most notably contraction in muscle and the activation of transcription in nuclei. Calcium for these events may come from outside the cell, or from intracellular compartments. Ryanodine receptors, or RyRs, are channels that rapidly release calcium from the stores. However, calcium delivered by this system activates contraction but does not affect events in the nucleus. Instead, calcium released from the stores by a different channel, the InsP3 receptor, activates transcription of DNA to RNA within the nucleus. A fundamental aspect of InsP3-mediated signaling is that calcium is released in a graded and slow manner in response to incremental levels of stimuli. In addition, InsP3 receptors are located in the nuclear envelope. The combination of these two factors is presumably responsible for the fact that nuclei seem to sense calcium liberated from the InsP3 receptor but not that released by RyRs. We have developed a unique way of detecting InsP3 in the nuclear envelope and plan to use this to elucidate changes in levels and types of these receptors that occur in dystrophic muscle.

Michael Granato Ph.D.

(RG) Zebrafish models of motor neuron and peripheral nerve diseases

\$133,391	7/1/2009	6/30/2010	Year 1
\$99,827	7/1/2010	6/30/2011	Year 2
\$104,082	7/1/2011	6/30/2012	Year 3

Summary Control of body musculature requires the interplay between the nervous system and the body musculature. Motor neurons form the 'interface' as their axons connect the nervous system with the muscle. Breakdown, degeneration and loss of motor axons is a hallmark of many hereditary neuropathies including Charcot-Marie-Tooth (CMT) disease, and motoneuron diseases such as Amyotrophic Lateral Sclerosis. Thus, axonal loss is a key determinant of clinical disability in hereditary neuropathies, and prevention of axonal loss is one of the most important therapeutic strategies currently being considered. To develop successful therapeutic strategies, it is therefore essential to focus on the events at the onset of the

disease, including gradual axonal loss or degeneration. Because it essential but technically very difficult to visualize this process inside animals, the goal of this proposal is to use the transparent zebrafish as a model in which to visualize and genetically characterize the axonal degeneration process. The optical transparency of the larvae and the relatively simple nervous system make this an outstanding model organism. Understanding the process of axonal degeneration will provide a powerful platform to directly test new therapeutic compounds to attenuate axonal degeneration and motor neuron loss.

Brett Anthony Kaufman Ph.D.

(DG) Identification and characterization of genes affecting mitochondrial myopathies

\$45,000 1/1/2010 12/31/2010 Year 3

Summary I am striving to identify genes involved in the normal and biased transmission of the mitochondrial genome. I have identified the mitochondrial protein TFAM, a known transcription factor, as having a direct role in the coordination and compaction of multiple mtDNAs together. Because compaction is essential to the transmission of mtDNA, our studies will garner insights into the causes of mtDNA-related diseases, which may in turn lead to therapeutic interventions for affected patients.

Todd Lamitina Ph.D.

(RG) Evolutionarily conserved synaptic function of dyferlin in *C. elegans* and mice

\$100,000 7/1/2009 6/30/2010 Year 1
\$100,000 7/1/2010 6/30/2011 Year 2
\$100,000 7/1/2011 6/30/2012 Year 3

Summary How and why mutations in dysferlin cause Limb-Girdle Muscular Dystrophy type 2B, or Dysferlinopathy, is poorly understood. The small model worm *C. elegans* expresses a Dysferlin gene that is highly similar to human Dysferlin and has nerves and muscle that are remarkably similar to their human counterparts. We have discovered that mutations in *C. elegans* Dysferlin cause defects in the communication between neurons and muscle. We have also discovered that similar defects are present in the muscle of Dysferlin mutant mice, suggesting that the function(s) of Dysferlin are evolutionarily conserved between *C. elegans* and mammals. Interestingly, similar defects underlie other muscle disorders, but a role for Dysferlin in muscle-neuron communication has never been proposed. Such defects are amenable to therapies that might prove beneficial to LGDM2B patients. In this proposal, we will 1) define the molecular role of Dysferlin in *C. elegans*, 2) test mechanisms for how Dysferlin may regulated muscle-neuron communication in worms and mice and 3) thoroughly characterize the synaptic defects caused by Dysferlin mutations in mammalian muscle. Our studies have the potential to define a new mechanism by which mutations in Dysferlin cause LGMD2B and may help to define a new treatment strategy for this disease.

Eran Perlson Ph.D.

(DG) Axonal transport alterations disrupt NMJ structure and function in ALS

\$45,000 1/1/2010 12/31/2010 Year 3

Summary Recent studies in animal models have shown that defects in axonal transport result in progressive neurodegenerative disease. The studies proposed here will characterize the role of those signaling factors in NMJ structure and maintenance as well as on nerve degeneration. We expect that the characterization

of those signals will provide novel insights into mechanisms of NMJ destabilization and neurodegeneration as well as provide a molecular basis for therapies and drug delivery.

Philadelphia - Thomas Jefferson University

Ya-Ming Hou Ph.D.

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

\$108,720	1/1/2010	12/31/2010	Year 1
\$108,720	1/1/2011	12/31/2011	Year 2
\$108,720	1/1/2012	12/31/2012	Year 3

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

Dena Jacob Ph.D.

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

\$60,000	7/1/2010	6/30/2011	Year 1
\$60,000	7/1/2011	6/30/2012	Year 2
\$60,000	7/1/2012	6/30/2013	Year 3

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

TENNESSEE

Memphis - St. Jude Children's Res. Hosp.

Brian David Freibaum Ph.D.

(DG) Characterizing the role of TDP-43 in ALS

\$60,000	7/1/2010	6/30/2011	Year 1
\$60,000	7/1/2011	6/30/2012	Year 2
\$60,000	7/1/2012	6/30/2013	Year 3

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

J. Paul Taylor M.D., Ph.D.

(RG) The molecular pathogenesis of spinobulbar muscular atrophy

\$95,000	1/1/2010	12/31/2010	Year 3
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Summary With prior MDA funding we developed a *Drosophila* model SBMA. We used this model to 1) characterize the roles of protein degradation pathways in disease and 2) discover a gene that rescues neurodegeneration when over-expressed. In this study we will 1) test our hypothesis that aberrant interaction of the disease protein with other transcription factors contributes to disease, 2) evaluate the therapeutic potential of a new class of drugs called SARMs for SBMA, and 3) perform a genetic screen to identify heretofore unrecognized genes involved in the disease.

Nashville - Vanderbilt University

Jun Li M.D., Ph.D.

(RG) Neurodegeneration in loss of function of Fig4

\$95,000	1/1/2010	12/31/2010	Year 2
\$95,000	1/1/2011	12/31/2011	Year 3

Summary We have recently described a novel recessively inherited disease, Charcot-Marie-Tooth type 4J (CMT4J) that is caused by mutations in the Fig4 gene. This mutation eliminates the expression of Fig4 in mice, namely pale temor (plt) mice that manifest weakness, neuronal loss and excessive vacuoles (like tiny membrane-bound 'bubbles' when visualized under the microscope) in neurons. Our subsequent studies in the plt mice and patients with CMT4J showed severe axonal loss and impaired trafficking of organelles (small cargos in the cells) in the mutant cells. These findings suggest that Fig4 is essential for neuronal survival. Moreover, we found a robust increase of mTOR activity in plt mice. Increased mTOR activity is

known to play an important role in some neurodegenerative disorders. In this investigation, we will first determine whether mTOR inhibitor protects neurons from degeneration in plt mice. Second, we will evaluate whether a combined treatment of lithium and inositol reduces vacuoles and restores organelle trafficking in Fig4 deficient cells. Therefore, this project may provide important insights into the pathogenesis of neuronal degeneration and potential therapeutic targets.

Allison Limpert Ph.D.

(DG) Regulation of NFkB activity during Schwann cell myelination

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary Defects in Schwann cell proliferation and myelination characterize several forms of Charcot Marie Tooth (CMT) disease. Ligands of the Neuregulin (NRG) family are well characterized axonal signals known to regulate Schwann cell proliferation and differentiation by binding to ErbB receptors on Schwann cells. While axons can express multiple NRG isoforms, these different ligands activate the same ErbB receptor complex on Schwann cells; however, the stimulation of these same receptors can result in varying biological outcomes. Correspondingly, we report that activation of ErbB receptors by a membrane bound, but not a soluble NRG isoform allows for the activation of NFkB, a transcription factor required for Schwann cell myelination. We hypothesize that the activation of this transcription factor by membrane bound NRG is a crucial event which promotes Schwann cell differentiation over proliferation. We propose to investigate the mechanisms by which NRG isoforms regulate NFkB during Schwann cell development and evaluate NRG signaling in mouse models of CMT. This will allow us to determine whether this signaling pathway can be used as a potential therapeutic target for demyelinating disorders.

TEXAS

Dallas - UT Southwestern Medical Center

Steve Cannon M.D., Ph.D.

(RG) A mouse model of hypokalemic periodic paralysis type 2

\$95,000	7/1/2009	6/30/2010	Year 3
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Summary Researchers will develop a mouse model of periodic paralysis to understand the ion channel functions in these disorders.

Steve Cannon M.D., Ph.D.

(RG) Mechanistic studies on the pathophysiology of hypokalemic periodic paralysis

\$52,180	7/1/2009	6/30/2010	Year 1
\$26,428	7/1/2010	6/30/2011	Year 2
\$26,428	7/1/2011	6/30/2012	Year 3

Summary Periodic paralysis is a rare disorder of skeletal muscle wherein affected individuals have recurrent attacks of severe weakness, lasting for hours to days. These episodes are caused by transient loss of muscle electrical excitability. In addition, some patients develop a late-onset permanent weakness in the legs that may prevent independent ambulation. The gene defect underlying the most commonly occurring form of periodic paralysis was identified 15 years ago, and yet we still do not understand how mutations in this calcium channel lead to a transient loss of muscle excitability. To gain further insight on the mechanistic basis for

periodic paralysis, we have developed an animal model by generating mice that have the analogous mutation of the skeletal muscle calcium channel gene that causes the most common form of human hypokalemic periodic paralysis. The studies in this proposal will characterize the extent to which the mouse model emulates the human disease, will define the link between altered function of mutant calcium channels and disruption of muscle excitability, and will enable us to study the mechanism by which drugs found empirically to be of partial benefit act and thereby promote a more systematic approach toward improving therapy in these debilitating disorders.

Jeffrey Leigh Elliott M.D.

(RRG) Animal models in ALS

\$260,626 11/1/2009 10/31/2010 Year 1

Summary This project will generate a mouse model of ALS which can be used to study ALS disease progression and therapeutic modalities. The transgenic mouse would express in the CNS a protein (TDP-43) linked to sporadic ALS.

Jeffrey Leigh Elliott M.D.

(RRG) The role of sumoylation in regulating nucleo/cytoplasmic trafficking of TDP-43

\$239,038 6/1/2010 5/31/2011 Year 1

Summary Changes in the sub-cellular localization of TDP-43 are a characteristic of sporadic ALS and familial ALS related to mutations in TDP-43. Normally, TDP-43 is found in the nucleus of cells. However, in ALS patients there is a shift toward increased amount of TDP-43 in the cytoplasm. Because this shift in TDP-43 localization appears to correlate with disease, it becomes important to understand the factors which regulate the transit of TDP-43 between nuclear and non-nuclear (cytoplasmic) compartments. Such insights may provide clues into potential disease mechanisms and most importantly avenues for targeted ALS therapies. In this project, we wish to determine whether a process known as sumoylation regulates the transport of TDP-43 between nucleus and cytoplasm. Sumoylation is the addition of a SUMO "group" to a specific sequence of a target protein which can then regulate the shuttling of the target protein between nucleus and cytoplasm. Human TDP-43 has such a SUMO recognition sequence and thus may be regulated by sumoylation.

Ronald Haller M.D.

(RG) Impaired oxidative capacity in McArdle disease: causes and treatment

\$127,700 1/1/2010 12/31/2010 Year 1
 \$127,700 1/1/2011 12/31/2011 Year 2
 \$127,700 1/1/2012 12/31/2012 Year 3

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

Woodring Wright M.D., Ph.D.

(RG) Immortal cells for myoblast transfer therapy

\$95,000 1/1/2010 12/31/2010 Year 3

Summary We have successfully immortalized adult skeletal myoblasts by introducing telomerase, an enzyme that prevents the telomere shortening that is normally used to count cell divisions. Our long-term goal is to create a universal donor that expresses molecules that prevent the cells from dying during the immediate post-transplantation period, maintains them transiently in a proliferative and migratory state, and which express dystrophin and factors that stimulate muscle hypertrophy.

Galveston - The University of Texas Medical Branch at Galveston

Premkumar Christadoss M.B.B.S

(RG) Innate immunity in autoimmune myasthenia gravis pathogenesis

\$118,750 1/1/2010 12/31/2010 Year 2

\$118,750 1/1/2011 12/31/2011 Year 3

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

Houston - Baylor College of Medicine

Hasan Orhan Akman Ph.D.

(DG) Generation and characterization of a mouse model of polyglucosan body disease

\$58,039 7/1/2009 6/30/2010 Year 2

\$59,246 7/1/2010 6/30/2011 Year 3

Summary The synthesis of glycogen is catalyzed by the sequential actions of two enzymes: (i) glycogen synthetase, which "strings" glucose to form linear chains to a length of approximately 10 glucose "beads"; and (ii) the branching enzyme, which attaches a short branch of approximately 4 glucose units to a linear chain in an alpha-1,6-glucosidic bond. Glycogen storage disease (GSD) type IV (OMIM 232500) is an autosomal recessive disorder caused by deficiency of the glycogen branching enzyme (GBE), and leads to the accumulation of an abnormal amylopectin-like polysaccharide in multiple tissues, including liver, heart, skeletal muscles, and central nervous system (CNS). A late-onset clinical variant, known as adult polyglucosan body disease (APBD), causes a neurodegenerative disorder simulating amyotrophic lateral sclerosis (ALS, Lou Gehrig disease), but often associated with bladder dysfunction and dementia. The mutated gene, GBE1, has been mapped to chromosome 3p14. The two naturally occurring animal models of this disorder, American quarter horses and Norwegian forest cats, are not practical laboratory animals. Therefore, we propose to develop a mouse model of GSD IV, which would be invaluable to better understand the pathogenesis of the disease and to test therapeutic strategies aimed at increasing the residual activity of branching enzyme in tissues.

Thomas A. Cooper M.D.

(RG) Oligonucleotide-based therapy for myotonic dystrophy				
\$115,354	1/1/2010	12/31/2010	Year 1	
\$112,794	1/1/2011	12/31/2011	Year 2	
\$112,794	1/1/2012	12/31/2012	Year 3	

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

Maria de Haro Ph.D.

(DG) Novel genes suppress CUG-induced muscle wasting				
\$60,000	1/1/2010	12/31/2010	Year 1	
\$60,000	1/1/2011	12/31/2011	Year 2	
\$60,000	1/1/2012	12/31/2012	Year 3	

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

Susan Hamilton Ph.D.

(RG) The role of FKBP's in skeletal muscle function and disease				
\$90,531	7/1/2009	6/30/2010	Year 2	
\$115,670	7/1/2010	6/30/2011	Year 3	

Summary FKBP's are immunophilins that regulate skeletal muscle excitation contraction coupling. We have strong evidence that one immunophilin, FKBP12.6,

can slow muscle fatigue and enhance recovery from injury. We now want to define the molecular mechanisms for these effects and use this knowledge to develop interventions that will slow fatigue and enhance recovery from muscle injury.

Muge N. Kuyumcu-Martinez Ph.D.

(DG) The mechanism of Protein Kinase C induced pathogenesis in myotonic dystrophy 1

\$60,000	7/1/2009	6/30/2010	Year 2
\$60,000	7/1/2010	6/30/2011	Year 3

Summary Regulation of signaling pathways is tightly controlled in response to stimuli, and inappropriate activation can lead to disease. Our studies have recently shown that prolonged activation of Protein Kinase C (PKC) signaling is important in Myotonic Dystrophy 1 (DM1). This activation leads to a change in the protein, CUG binding protein 1 (CUGBP1), such that the protein becomes phosphorylated, leading to its increased levels in heart tissue from individuals with the disease. Higher levels of CUGBP1 have been implicated in the cause of DM1 and these results explain the basis for this increase. Inhibition of PKC signaling by specific inhibitors in a DM1 heart-specific mouse model prevents mortality of the mice and reduce phosphorylation and steady state of CUGBP1 protein levels. The goal of my research is to understand what role PKC plays in the disease and to explore PKC inhibitors as a therapy option for DM1 patients. The findings from heart model may provide insights for alleviating the severe skeletal muscle phenotype seen in DM1 patients.

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

(RG) Role of abnormal calcium homeostasis in cardiac disease in muscular dystrophy

\$98,612	1/1/2010	12/31/2010	Year 3
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Summary Dystrophin deficiency in the heart causes abnormalities in calcium signaling, which in turn cause heart failure and fatal arrhythmias. Using biopsies from patients with MD and a mouse model of dystrophin deficiency, the role of abnormal calcium release in heart muscle cells will be investigated.

Lee-Jun C. Wong Ph.D.

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

\$137,000	7/1/2010	6/30/2011	Year 1
\$137,000	7/1/2011	6/30/2012	Year 2
\$137,000	7/1/2012	6/30/2013	Year 3

Summary Mitochondria are the cellular organelles where energy currency, ATP, is produced. Diagnosis of mitochondrial disorder is very difficult because the disease itself can present in many different forms, including muscle weakness, exercise intolerance, ophthalmoplegia, sensorineural deafness, seizures, ataxia, movement disorder, and stroke like episodes. In addition to the small mitochondrial genome, as many as 1500 nuclear-encoded proteins are targeted to mitochondrion. Molecular defects in any of these genes can potentially cause mitochondrial disorder, which predominantly has neuromuscular features. Currently, the diagnosis is based on the gold standard of sequence analysis gene by gene. It is tedious, expensive, time consuming, and is only for the detection of point mutations. Other methods will have to be used for the detection of large deletions and copy number changes. We propose to establish a one-step novel technology that would allow us to detect point mutations and deletions in both nuclear and mitochondrial genomes,

with simultaneous detection and estimation of heteroplasmic mitochondrial DNA point mutations or deletions, as well as mitochondrial DNA depletion. The availability of a one-step diagnostic approach is particularly important since mitochondrial myopathy accounts for a large proportion of patients, from children to adults, with muscular dystrophy. Prompt definitive diagnosis is essential for proper patient management, treatment, and genetic counseling.

Houston - Methodist Neurological Institute

Stanley Appel M.D.

(RG) Immune mechanisms in amyotrophic lateral sclerosis

\$95,000 7/1/2009 6/30/2010 Year 3

Summary Studies will define protective or toxic properties of immune cells in disease, particularly ALS.

Stanley Appel M.D.

(CRNG) Clinical Research Network Grant

\$100,000 8/1/2009 7/31/2010 Year 2

\$100,000 8/1/2010 7/31/2011 Year 3

Summary We hypothesize that ALS patients with hyperlipidemia progress slower and have longer survival than those with normal or low lipid levels. Furthermore, these patients have more stable nutrition and metabolism throughout the disease, but also a greater prevalence of cardiovascular disease risk factors and associated indices of inflammation. We propose a three year, multicenter, prospective study of blood lipid profiles in ALS and its relation with disease progression and survival, in addition to other co-variables disease.

Stanley Appel M.D.

(RG) Immune mechanisms in amyotrophic lateral sclerosis

\$110,000 7/1/2010 6/30/2011 Year 1

\$110,000 7/1/2011 6/30/2012 Year 2

\$110,000 7/1/2012 6/30/2013 Year 3

Summary Neuroinflammation is a pathological hallmark in amyotrophic lateral sclerosis (ALS), and is characterized by activated microglia and infiltrating T cells at sites of neuronal injury. In ALS, neurons do not die alone; neuronal injury is non cell-autonomous and depends on a well-orchestrated dialogue in which neuronally secreted misfolded proteins activate microglia and initiate a self-propagating cycle of neurotoxicity. Diverse populations and phenotypes of CD4+ T cells crosstalk with microglia, and depending on their activation status, influence this dialogue and promote neuroprotection or neurotoxicity. A greater understanding of the T cell population that mediates these effects, as well as the molecular signals involved should provide targets for neuroprotective immunomodulation to treat this devastating neurodegenerative disorder

Houston - The University of Texas Health Science Center at Houston

Rebecca Berdeaux Ph.D.

(DG) The role of CREB in skeletal muscle regeneration and hypertrophy

\$45,000 1/1/2010 12/31/2010 Year 3

Summary The proposed studies are designed to explore the cellular and physiological consequences of over-activation of CREB in cultured muscle cells and

in the muscle of mice. We will test whether over-activation of CREB can also improve muscle of mice with muscular dystrophy. Through these studies, we hope to determine how this DNA binding protein may control the balance between muscle growth and muscular dystrophy.

Vihang A. Narkar Ph.D.

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

\$111,822	7/1/2010	6/30/2011	Year 1
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\$95,252	7/1/2011	6/30/2012	Year 2
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\$95,252	7/1/2012	6/30/2013	Year 3
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Summary One emerging strategy to treat Duchenne muscular dystrophy is to increase aerobic muscles that have enhanced oxidative capacity and resistance to fatigue. Therefore, discovery of regulatory factors that increase aerobic muscles is of paramount importance in treating DMD. We have found that over-expression of ERRgamma by genetic engineering in mouse skeletal muscle activates genes that encode a highly aerobic and fatigue resistant muscle, suggesting that targeting this protein might be beneficial in muscular dystrophy. The current project is designed to investigate the extent to which ERRgamma rescues the pathology of DMD by increasing aerobic fatigue-resistant muscles. We plan to achieve this by genetically activating ERRgamma in the skeletal muscles of mdx mice, a rodent model of DMD, and measuring the ameliorative effects of the protein in restoring muscle architecture and performance. Our research has future implications in designing novel therapies to treat DMD by remodeling the muscle type.

Irina I. Serysheva Ph.D.

(RG) Structural studies of skeletal muscle RyR channel

\$115,988	7/1/2009	6/30/2010	Year 1
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\$117,802	7/1/2010	6/30/2011	Year 2
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\$118,945	7/1/2011	6/30/2012	Year 3
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Summary Numerous physiological processes are controlled by Ca²⁺, including muscle contraction and heartbeat. Intracellular Ca²⁺ levels are regulated by 'Ca channels', specialized proteins that allow Ca²⁺ to pass through cell membranes. Dysfunction of these channels leads to a wide array of diseases, such as muscle and cardiovascular disorders. Many drugs currently in use to treat these diseases target Ca²⁺ channels. Due to high prevalence of muscle disorders in the USA and worldwide, new ways to interfere with Ca²⁺ channels in disease states are needed. Design of such strategies, however, is hampered by the lack of sufficient knowledge about the architecture of these proteins. In this particular project, we study the type 1 ryanodine receptor (RyR1), the main Ca²⁺ release channel in skeletal muscle. RyR1 mediates Ca²⁺ release from the sarcoplasmic reticulum to initiate muscle contraction. Our major approach consists of electron cryomicroscopy, a technique that allows us to elucidate the structure of a protein at subnanometer resolution. The resulting structures are excellent templates for the design of novel drugs, and provide invaluable information about how RyR1 works. We also assess RyR1 function using radioligand binding assays and measuring Ca²⁺ fluxes through channels reconstituted into lipid vesicles. By combining these techniques, we aim to increase our understanding of Ca²⁺ release channels, laying the basis for novel strategies to interfere with their function in disease.

Ralf Krahe Ph.D.

(RG) A transgenic mouse model for myotonic dystrophy type 2 (DM2)
\$123,500 7/1/2009 9/30/2010 Year 3

Summary Investigators will study a mouse model to determine effect of DM2 expansion on development as well as other stages.

San Antonio - Univ of Texas Health Science Center at San Antonio**Holly Van Remmen Ph.D.**

(RG) PLA2 related inflammatory pathways in the pathogenesis of ALS
\$118,750 7/1/2009 6/30/2010 Year 2
\$118,750 7/1/2010 6/30/2011 Year 3

Summary In this study, we will examine the role of inflammation in neuronal and muscular degeneration in ALS. We will determine the role of the enzyme phospholipase A2 (PLA2) and inflammatory mediators in inducing loss of innervation in muscle and effects on mitochondrial dysfunction and muscle atrophy using mouse models of ALS bred to knockout mice lacking PLA2. We will also determine the ability of anti-inflammatory agents to alter neuromuscular degeneration to better understand role of inflammatory pathways in ALS, hopefully to lead to new interventions.

UTAH**Salt Lake City - Sfida BioLogic, Inc.****John Paul Manfredi Ph.D.**

(RG) Evaluation in zebrafish of small molecules as SMA therapeutics
\$63,257 7/1/2010 6/30/2011 Year 1
\$16,020 7/1/2011 6/30/2012 Year 2

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

Salt Lake City - University of Utah**Russell J. Butterfield M.D., Ph.D.**

(CRTG) Natural history and genetic epidemiology of collagen VI related myopathies
\$86,000 7/1/2010 6/30/2011 Year 2

Summary Congenital muscular dystrophies are a group of disorders resulting in muscle weakness. Patients with Ullrich congenital muscular dystrophy (UCMD) have early onset of severe weakness, contractures, and hyper-elasticity of joints. Bethlem myopathy (BM) is a milder syndrome with slowly progressive weakness and contractures in the joints of the hands with onset in the childhood or adolescence. Both BM and UCMD are caused by mutations in the genes that produce collagen VI. Once thought to be rare clinical entities, collagen VI related myopathies are now considered among the most common causes of congenital muscle weakness. Multiple mutations in the genes producing collagen VI have been reported including 75 unique variants. The Genome Center at the University of Utah has developed a method for rapid and complete sequencing of the collagen VI genes and has evaluated approximately 135 patients. Despite the growing pool of patients with genetic testing, clinical information is lacking. Our proposal is to develop a database housing both clinical and genetic information on patients with collagen VI related myopathies. Having both clinical and genetic data on these patients, we will be able to study relationship between the particular genetic mutation, and the manifestation of disease. We expect that the database will be a resource to investigators studying collagen VI related disorders and provide a pool of patients available for clinical trials and other studies.

Michael Therron Howard Ph.D.

(RG) Antisense mediated suppression of DMD frameshift mutations

\$123,500	1/1/2010	12/31/2010	Year 3
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Summary The goal of this study is to develop a novel antisense oligonucleotide based approach for treating Duchenne Muscular Dystrophy patients with frameshift mutations in the DMD gene. We will test the ability of antisense oligonucleotides to alter protein synthesis in a controlled way to suppress the effects of frameshift mutations. As this approach is generally applicable to frameshift mutations in any gene, the therapeutic strategy developed here may be adapted for the treatment of many different genetic diseases of the muscle and nervous systems.

Gabrielle Kardon Ph.D.

(RG) Role of connective tissue fibroblasts in muscle regeneration

\$118,569	7/1/2009	6/30/2010	Year 1
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\$148,942	7/1/2010	6/30/2011	Year 2
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\$146,418	7/1/2011	6/30/2012	Year 3
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Summary Duchenne Muscular Dystrophy (DMD) is characterized by pathological muscle degeneration and regeneration and connective tissue fibrosis. Muscle regeneration is mediated by resident stem cells called satellite cells. In DMD repeated rounds of regeneration lead to the diminished proliferation and regenerative capacity of the satellite cells and ultimately to the loss of muscle mass and function. Concurrent with the loss of muscle mass there is an excessive increase in muscle connective tissue, termed fibrosis, which further impairs muscle function. We propose that the fibroblasts embedded in the connective tissue contribute significantly to the pathology of DMD. These fibroblasts may regulate satellite cells and are likely to be the source of connective tissue fibrosis. Study of these connective tissue fibroblasts has been severely hampered by the lack of molecular markers for these cells. Recently we have identified the gene Tcf4 as a robust marker of these fibroblasts. We propose to use mouse genetics to test the role of Tcf4+ connective tissue fibroblasts in regulating satellite cells and fibrosis during muscle regeneration. In addition, we will test the role of an important

signaling molecule, β -catenin, in satellite cells, fibroblasts, and muscle cells in muscle regeneration. Results from our research will give us insights into the cell-cell and cell-connective tissue interactions governing normal muscle regeneration and the pathology of DMD.

Kathryn J. Swoboda M.D.

(RG) Phase I/II study of NaPB in pre-symptomatic infants with SMA: STOP SMA STUDY

\$118,159	1/1/2010	12/31/2010	Year 2
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\$103,331	1/1/2011	12/31/2011	Year 3
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Summary Most babies with SMA are normal or nearly normal as newborns. However, babies who are at risk to develop SMA type I or type II typically develop significant weakness in the first few weeks or months of life. STOP SMA is a clinical trial designed to assess safety and effectiveness of sodium phenylbutyrate (NAPB) in infants confirmed to have SMA by genetic testing. Eligible infants are those likely to develop either type I or type II SMA because they have an affected older sibling and have tested positive for SMA via genetic testing. Babies predicted to develop SMA type I must be enrolled by 3 months of age, and babies predicted to develop SMA type II must be enrolled by 6 months of age. All babies will receive study drug, and we will assess safety and possible effectiveness by comparing them to children in our natural history database.

VIRGINIA

Charlottesville - University of Virginia

Mani S. Mahadevan M.D.

(RG) Insights into RNA toxicity in DM1

\$104,500	7/1/2009	6/30/2010	Year 3
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Summary Investigators will utilize a new mouse model of RNA toxicity for DM1 to study modulation of Nkx2.5 seen to be important in cardiac effects in DM1.

Mani S. Mahadevan M.D.

(RG) Modifiers of RNA toxicity in DM1

\$151,138	1/1/2010	12/31/2010	Year 2
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\$157,054	1/1/2011	12/31/2011	Year 3
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Summary Myotonic muscular dystrophy type 1 (DM1) is caused by an expanded (CTG) tract in the 3' untranslated region of the DM protein kinase (DMPK) gene. DM1 is the prototype for a mutation that results in the production of a toxic RNA. The toxic RNA is thought to mediate its effects through RNA-binding proteins, primarily Muscleblind (MBNL1) and CUG-binding protein (CUGBP1), both of which are proteins involved in a process called RNA splicing (in which RNAs are "cut and pasted" together). MBNL1 is believed to be sequestered from its normal function by the mutant DMPK RNA and CUGBP1 levels are elevated. The result is aberrant splicing of target RNAs. It has been shown that increasing MBNL1 levels alleviates myotonia, a key feature of DM1. But reducing CUGBP1 levels has not been studied. We will use our novel, inducible mouse model of RNA toxicity in DM1 to study the effects of reducing CUGBP1. This will be done using three strategies: 1) breeding our mice with mice having reduced amounts of CUGBP1, 2) breeding our mice with mice deficient in enzymes that are thought to be responsible for increasing CUGBP1 levels, and 3) treating our mice with drugs known to inhibit those enzymes. The

mice will be analyzed to see if this reduces the RNA toxicity. Our goal is to characterize the contribution of elevated CUGBP1 to DM1 pathogenesis and identify possible therapeutic modalities to reducing CUGBP1 in DM1.

Ramesh S. Yadava Ph.D.

(DG) Development of RNA-based therapy for myotonic dystrophy (DM1)

\$60,000 7/1/2009 6/30/2010 Year 2

\$60,000 7/1/2010 6/30/2011 Year 3

Summary Myotonic dystrophy (DM1) is the most common form of inherited muscular dystrophy in adults, with a prevalence of 1 in 8,000. It is a multi-system disorder that affects skeletal muscles, smooth muscles the heart, eyes, and endocrine and central nervous systems. At present, there is no therapy for myotonic dystrophy. The overall aim of this proposal is to develop RNA-based therapeutics to selectively destroy the toxic mutant DMPK mRNA that causes DM1. We plan to screen a large number of RNA molecules (antisense RNAs, shRNAs and ribozymes) for silencing the mutant DMPK mRNA using cell culture system, and test their efficacy in an inducible mouse model of RNA toxicity that was developed in our lab. The ultimate goal is to generate novel therapeutic agents capable of selectively eliminating the toxic RNA in DM1 patients.

WASHINGTON

Seattle - Fred Hutchinson Cancer Research Center

Galina Filippova Ph.D.

(RG) Role of chromatin structure in FSHD

\$137,384 1/1/2010 12/31/2010 Year 3

Summary This study will identify chromatin changes specific to FSHD associated D4Z4 repeats and test the hypothesis that these changes alter the binding of a chromatin insulator factor, CTCF, as the interaction of this region with other regions in the genome. We anticipate that this study will contribute to our understanding of the epigenetic mechanisms underlying FSHD. Moreover, the identified epigenetic mechanisms involving higher order chromatin organization will provide new insights in the complex etiology of other genetic disorders.

Maura H. Parker Ph.D.

(DG) Myogenic cell transplant in an immune-tolerant canine model of DMD

\$45,000 7/1/2009 12/31/2010 Year 3

Summary Researchers have induced immune tolerance in GRMD dog model for DMD and will access methods to affect myogenic stem cell repair in this model.

Zejing Wang M.D., Ph.D.

(DG) The immunological barrier to AAV-mediated gene therapy in a canine model of DMD

\$60,000 1/1/2010 12/31/2010 Year 2

\$60,000 1/1/2011 12/31/2011 Year 3

Summary Adeno-associated virus (AAV)-mediated micro-dystrophin delivery to skeletal muscle has been successful in restoring muscle function in dystrophic mice. However, recent human studies indicated that the efficacy of AAV-mediated therapies is limited by immune responses to viral capsid proteins. Also, we demonstrated robust cellular immune responses to AAV capsid proteins after direct

intramuscular injection of AAV vectors in wild type and DMD dogs. We further demonstrated that immune responses to AAV vectors could be averted by a brief course of immunosuppression. We will develop and validate assays to better characterize immune responses to AAV vectors and transgenes in dogs, and develop efficient and non-toxic strategies to induce immunological tolerance to these immunogens using newly developed blockers of T-lymphocyte activation and T-cell regulatory molecules. We anticipate that results of our work in dogs can be directly translated to DMD clinical trials.

Seattle - University of Washington

Glen B. Banks Ph.D.

(DG) Skeletal muscle replacement in mdx mice with muscle stem cells

\$60,000	1/1/2010	12/31/2010	Year 1
\$60,000	1/1/2011	12/31/2011	Year 2
\$60,000	1/1/2012	12/31/2012	Year 3

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

Jeffrey S. Chamberlain Ph.D.

(RRG) Systemic delivery of AAV vectors to muscle

\$415,431	10/1/2008	5/1/2010	Year 1
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Summary Gene therapy would be an ideal solution for Duchenne muscular dystrophy. The ability to replace the broken gene in human muscles is a critical goal. Finding a way to deliver the gene to each and every muscle cell in the body, having the gene work, getting the therapy to last long term, and finding a way to reverse damage which has already occurred are all major components to curing this disease. Our group was the first to show that new genes can be delivered to all the muscles of an adult animal. Our previous studies show we can essentially cure DMD in mice by whole body delivery of a new dystrophin gene carried in a delivery shuttle vector known as AAV. This application is to follow-up preliminary data showing that the method might be scaled-up, and to determine the feasibility of bringing this treatment method into the clinic.

Joel R. Chamberlain Ph.D.

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

\$100,000 4/1/2010 3/31/2011 Year 1

\$100,000 4/1/2011 3/31/2012 Year 2

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

Stephen D. Hauschka Ph.D.

(RG) Regulatory cassettes for treating diseased cardiac muscle

\$102,260 7/1/2009 6/30/2010 Year 1

\$105,311 7/1/2010 6/30/2011 Year 2

\$108,452 7/1/2011 6/30/2012 Year 3

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

Guy Leary Odom Ph.D.

(DG) Vectors to avoid a cellular immune response against dystrophin in DMD patients

\$60,000	1/1/2010	12/31/2010	Year 2
\$60,000	1/1/2011	12/31/2011	Year 3

Summary Dystrophin provides a structural link within muscle cells that protects them against damage during strain. One caveat for dystrophin replacement therapy in DMD patients is the possibility of the immune system seeing the newly introduced protein as foreign. Utrophin, a protein similar to dystrophin in structure and function is normally expressed in nearly all cells of the human body. Targeted striated muscle gene delivery of utrophin may provide the benefit of avoiding destructive cellular immune responses, thus improving the efficiency of gene transfer to DMD patients. We have recently designed and delivered a murine microutrophin cassette which shows a tremendous reduction in muscle abnormalities in muscular dystrophy mice that lack both dystrophin and utrophin (mdx/utrn-/-). We propose to characterize the functionality of human microutrophin in mdx/utrn-/- mice. We further propose to test AAV-mediated delivery of human microutrophin in non-human primates as part of preclinical studies aimed at developing gene therapy for DMD.

Leo Pallanck Ph.D.

(RG) Influence of the mitochondrial quality control system on mitochondrial myopathy

\$104,233	7/1/2010	6/30/2011	Year 1
\$104,233	7/1/2011	6/30/2012	Year 2
\$104,233	7/1/2012	6/30/2013	Year 3

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

Justin Percival Ph.D.

(DG) Reevaluation of nNOS isozyme function in mdx skeletal muscle

\$44,992	1/1/2010	12/31/2010	Year 3
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Summary We have identified a novel nNOS signaling pathway based on nNOSbeta, which appears to regulate muscle mass and strength. We propose to further characterize the function of the nNOSbeta signaling pathway in normal and mdx muscle and determine if increased nNOSbeta activity can improve the dystrophic phenotype of mdx skeletal muscle.

Zipora Yablonka-Reuveni Ph.D.

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

\$128,591	7/1/2009	6/30/2010	Year 1
\$131,781	7/1/2010	6/30/2011	Year 2
\$135,069	7/1/2011	6/30/2012	Year 3

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

WISCONSIN

Madison - University of Wisconsin

F. Michael Hoffmann Ph.D.

(RG) New TGF-beta signaling inhibitors for activating muscle regeneration

\$118,750	1/1/2010	12/31/2010	Year 3
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Summary We intend to discover chemical agents that can inhibit the problems caused by excess TGF-beta on muscle regeneration but that do not interfere with the positive roles of TGF-beta. Initial screens have identified candidate chemical inhibitors that are being optimized for potency and selectivity. We will do additional screens of small molecule chemical libraries to identify chemicals that are selective for TGF-beta's affect on muscle regeneration. These compounds could provide initial leads for developing drugs that would activate muscle regeneration by inhibiting TGF-beta signaling.

Milwaukee - American Society of Gene Therapy

Mary Dean BA

(SG) Expanding Frontiers in Spinal Muscular Atrophy Gene Therapy

\$3,361	5/19/2010	12/31/2010	Year 1
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Summary This symposium was an opportunity to have MDA work cooperatively with 3 other foundations with a focus on spinal muscular atrophy to develop a special symposium geared toward gene therapy researchers and other researchers with an interest in what is being done in the area of spinal muscular atrophy. This symposium is the second time that ASGCT has worked with non-profit foundations to develop an educational program for researchers.