August 8, 2012

THE ALS ASSOCIATION'S GLOBAL RESEARCH AWARDS 2012

The ALS Association's latest research awards include funding commitments of $4 million to scientists in 31 laboratories in the United States, United Kingdom, Belgium, Germany and Canada. These awards are part of its **T**ranslational **Re**search **A**dvancing **T**herapies for ALS (TREAT ALS™) portfolio, a diverse portfolio of amyotrophic lateral sclerosis (ALS) research to find treatments and a cure for Lou Gehrig's Disease. These new awards include Investigator-Initiated Awards, The Milton Safenowitz Post-Doctoral Fellowship for ALS Research Awards, and The ALS Association-Initiated Awards.

INVESTIGATOR INITIATED AWARDS

Investigators submit a proposed line of inquiry covering diverse areas of research, and The ALS Association convenes a scientific review committee to discuss proposals and make selections based on the merits of each. There are two types of Investigator-Initiated Awards: innovative discovery awards and multi-year awards.

Innovative discovery awards:
This year there are nine innovative discovery awards, which are aimed at: researchers just starting their careers in ALS research, established ALS investigators looking to fund pilot studies, or senior post-doctoral fellows proposing novel ideas for ALS-related projects.

Researcher: Brian Wagner, Ph.D., Children's Hospital; Boston, Massachusetts

Topic: Using Electrophysiology to study ALS patient-derived motor neurons
**Funded by The Alan Phillips Discovery Award*

Description: The primary function of a motor neuron is to collect and integrate signals from the brain and spinal cord and transmit an outgoing electrical signal that results in muscle contraction. The researchers hypothesize that investigating the electrical properties of healthy and diseased motor neurons will help increase understanding of ALS and yield insight into how to treat the disease. Specifically, using recent advances in stem-cell technology, the investigators will derive motor neurons from ALS and control subjects and study the electrical profile of the motor neurons. Preliminary evidence suggests that ALS motor neurons are hyperexcitable, meaning that they are prone to too much activity. This increased activity may exhaust the motor neurons and ultimately cause or contribute to their death, a hallmark finding in ALS. The proposed project consists of evaluating the hyperexcitability in both inherited and sporadic forms of ALS and identifying the

cellular mechanisms responsible for the hyperexcitability. Based on the detected mechanisms, the investigators will then test the effects of specific drugs that may help reduce the excitability in vitro and determine whether these drugs inhibit motor neuron death, which can then be tested in mouse surrogate disease models.

Researcher: Kevin Foust, Ph.D., Ohio State University; Columbus, Ohio

Topic: Bicistronic AAV9 Vectors for Mutant SOD1 knockdown and trophic support in ALS
**Funded by The Alan Phillips Discovery Award*

Description: ALS genetics has seen a recent boom with the discoveries of TDP43, FUS and C9orf72, as known causative agents along with SOD1. Translational strategies to modify these malfunctioning genes in the nervous systems of patients are important for ALS therapeutic development. Adenoassociated viral (AAV) gene delivery can be used to effectively modify gene expression levels within the CNS. AAV gene therapy has proven safe and effective in clinical trials for other genetic diseases. Recently, investigators demonstrated that AAV can enter the brain and spinal cord after an intravenous injection and infect cells important in ALS disease progression. The proposed study will use AAV gene delivery to reduce levels of mutant SOD1 expression while simultaneously producing extracellular factors that help support motor neurons. This multi-faceted approach can be modified to target other known ALS genes that play roles in both familial and sporadic ALS. The proposed study will treat ALS animals either before or after the onset of symptoms to assess the experimental treatment in a clinically applicable paradigm. It is expected that successful completion of these experiments will lay the groundwork for new clinical trials in ALS.

Researcher: Scott Phillip, Ph.D., University of Alabama at Birmingham; Birmingham, Alabama

Topic: Proteosome activity enhancement as treatment for ALS

Description: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the loss of motor neurons. Sporadic and familial forms of ALS display the formation of ubiquitin tagged aggregates suggesting they are not effectively eliminated by the ubiquitin proteasome system (UPS). One component of the UPS, Usp14, is perfectly positioned to regulate the degradation of ubiquitin tagged proteins. Genetic or pharmacological inhibition of Usp14's catalytic activity can accelerate the degradation of mutant aggregate forming proteins. The focus of this proposal is to determine if inhibition of Usp14's catalytic activity with already developed small molecule inhibitors can reduce the levels of aggregate-prone proteins in familial ALS. This novel approach of proteasomal acceleration for the degradation of ALS mutant proteins can be a powerful treatment option for individuals suffering from ALS.

Researcher: Kathryn Ivey, Ph.D., Gladstone Institutes; San Francisco, California

Topic: Interaction Between Neuromuscular Junctions Regulating Muscle-miRNA's and TDP43
**Funded by the California Chapters of The ALS Association*

Description: ALS causes muscle weakness and deterioration as a result of motor neuron death. Therefore, cell-based research into the molecular mechanisms underlying ALS focus largely on processes occurring in motor neurons. However, neural degeneration is preceded by a breakdown of neuromuscular junctions (NMJs), which are normally maintained in response to signals from both the muscle and motor neurons with which they are associated. This newly appreciated fact opens the possibility that deleterious processes occurring in skeletal muscle can contribute to the disease. The investigator has identified a unique physical interaction between a protein called TDP-43, whose dysfunction is known to cause ALS, and a class of related small RNAs, known as microRNAs (miRNAs), that are exclusively expressed in muscle cells. Deletion of one of these miRNAs from the mouse genome blocks efficient regeneration of neuromuscular junctions after acute nerve injury, especially in a mouse model of ALS. Therefore, she proposes to determine whether there is a link between TDP-43 function and miRNA activity in muscle cells and how this link may be disrupted in the setting of ALS, thereby contributing to the disease. Because miRNAs and miRNA-blocking molecules offer promise as potential therapeutics, insight into their contribution to disease processes is a major step toward developing new disease interventions.

Researcher: Steven Crone, Ph.D., University of Chicago; Chicago, Illinois

Topic: Degeneration of spinal circuitry in mouse models of ALS

Description: The proposed research seeks to understand the contribution of spinal interneurons to the onset and progression of disease in ALS. The activity and function of spinal motor neurons are dependent upon interneurons responsible for initiating and coordinating motor activity. How spinal interneurons are affected by ALS and how the function of interneurons impacts motor neuron survival and disease progression are largely unexplored questions. The investigator has previously demonstrated important roles for glutamatergic V2a interneurons in locomotor and respiratory function, two motor behaviors severely impaired in ALS patients. Preliminary investigations using the SOD1(G93A) transgenic mouse model of ALS demonstrate that spinal V2a interneurons degenerate prior to symptom onset. He has developed transgenic mice in which V2a excitatory synapses can be labeled and identified in order to determine if V2a synapses degenerate in mouse models of ALS. He will also alter SOD1 expression in V2a neurons or ablate V2a neurons to determine if changes in the number of V2a interneurons or their synapses influence motor neuron survival or disease progression in ALS mouse models. These experiments will assess the contribution of spinal interneurons to ALS and determine whether therapies that target interneurons would be beneficial to ALS patients.

Researcher: Renee Douville, Ph.D. University of Winnipeg; Winnipeg, Manitoba, Canada

Topic: Involvement of TDP43 Dysregulation in Eliciting Human Endogenous Retrovirus Activity in ALS

Description: Integrated into human DNA resides thousands of retrovirus-like sequences which comprise nearly 8% of the human genome. Investigators have recently shown that there is active human endogenous retrovirus (HERV-K) in neurons of patients with amyotrophic lateral sclerosis (ALS). Retroviruses can cause central nervous system damage before the onset of diagnosable symptoms. Human cells can actively counteract retrovirus replication when aided by viral restriction factors – proteins that are up-regulated by the innate immune response. TAR DNA-binding protein 43 (TDP-43) is a viral restriction factor which is known to be dysregulated in ALS; therefore, investigators plan to evaluate whether TDP-43 blocks the expression of HERV-K in healthy neurons but not the neurons of patients with ALS. We will also determine if HERV-K elicits innate immune and inflammatory responses in the neurons of patients with ALS. These findings will provide insight into therapeutic antiviral regimens which could prevent the onset of central nervous system damage and symptoms triggered by endogenous retrovirus activity in ALS.

Researcher: Allison Ebert, Ph.D., Medical College of Wisconsin; Milwaukee, Wisconsin

Topic: Generation and characterization of ALS IPSC derived skeletal muscle
**Funded by The ALS Association, Wisconsin Chapter*

Description: This project utilizes previously generated induced pluripotent stem cells (iPSCs) derived from a patient with the ALS8 mutation in VAPB in order to generate skeletal muscle and characterize neuromuscular junction (NMJ) properties. Motor neurons are routinely produced from iPSCs and assessed for disease phenotypes, but there has been no examination of the functional properties of ALS iPSC derived skeletal muscle. Although ALS8 iPSC derived motor neurons can form NMJs, their number, size, and distribution have not been evaluated in control or diseased muscle co-culture conditions. The investigators hypothesize that ALS8 iPSC derived skeletal muscle will negatively impact NMJ formation and function. The ALS8 iPSC line is ideal for the proposed pilot study, but these studies can be expanded to include additional genetic and sporadic forms of ALS. Data collected through this proposal may help determine the molecular signaling processes of mutant muscle leading to NMJ dysfunction and disease progression with the ultimate goal of developing more effective therapies.

Researcher: Thomas Jahn, Ph.D., German Cancer Research Center; Heidelberg, Germany

Topic: Modulation of protein aggregation in ALS

Description: The tendency to loose native structure and thereby misfold into alternative conformations is shared by most, if not all, polypeptide sequences. The complementary processes of protein misfolding and aggregation accompany, and may underpin the pathogenesis of, neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). With this discovery, grant investigators will develop novel tools aimed at identifying pathways that modulate the aggregation of the 43kDA transactive response DNA-binding protein (TDP-43), mutations of

which have recently been identified to account for more than 4% of ALS cases. The investigators intend to establish comprehensive tools using cell culture and *Drosophila* models to delineate loss-of-function and gain-of-toxicity processes for disease-associated variants of TDP-43. By combining in vitro and in vivo tools to quantitatively assay the misfolding and mislocalization of TDP-43, they will further analyze the downstream pathways activated by protein aggregation and ultimately leading to neurodegeneration. Most importantly, the tools generated here will provide a rational basis for the development of more detailed high-throughput studies and guide future approaches to probe the role of protein misfolding in ALS, opening new routes toward the design and development of rational treatments for this debilitating disease.

Researchers: Tracey Caller, Ph.D. and Elijah Stommel, M.D., Ph.D.; Dartmouth-Hitchcock Medical Center; Lebanon, New Hampshire

Topic: Epidemiology of ALS in Northern New England. A possible relationship to environmental toxins

Description: ALS is a devastating and fatal neurodegenerative disease for which there is no treatment. The majority of cases are not familial, but sporadic, and an environmental influence is likely to play a role in the development of ALS. Detailed epidemiology studies can be used to generate hypotheses for disease etiology. In the United States, the lack of quality databases has limited detailed epidemiologic investigations. The ALS Center at Dartmouth-Hitchcock Medical Center is collaborating with other providers and regional centers to develop a comprehensive ALS patient database to characterize the epidemiology of ALS within Northern New England. Their data suggest that ALS occurs at a higher frequency within certain locations, and environmental exposures could play a role in the geographic variation of disease incidence. They believe that by providing a detailed surveillance of ALS in their region, they can help further investigation of environmental influences in the development of ALS. Their preliminary data suggests that there are geographic disparities in development of ALS in their region. They have observed that areas of high ALS incidence seem to cluster around certain bodies of water, and they hypothesize that this could possibly be related to environmental factors. In particular, they are interested in both BMAA and methylmercury, two toxins that have been hypothesized in the literature to increase the risk of developing ALS, both of which are prominent in their regional water bodies. They will use a case-control study to evaluate potential exposure to cyanobacteria or water contaminants as a potential risk factor for ALS, while evaluating for other possible occupational and environmental risk factors that might contribute to neurodegeneration.

Multi-year awards include the following:
The multi-year awards involve a one to three year funding commitment from The ALS Association and are awarded to established ALS researchers proposing research highly relevant to ALS.

Researcher: John Ravits, Ph.D., University of California San Diego; La Jolla, California

Topic: Investigating transcriptome changes in hexanucleotide repeat expanded C9orf72 ALS

** Funded by The ALS Association, Greater Philadelphia Chapter*

Description: In October 2011, an important class of mutations called expansion repeats was identified in the c9orf72 gene in about 33% of familial ALS and about 4-7% of sporadic ALS, thus representing the single most common genetic cause of ALS. Essentially nothing is known about this gene or how the expanded repeat in it causes ALS. The two leading theories are that the expanded repeat alters the c9orf72 gene function and that the expanded repeat is somehow itself directly toxic to the cells. In this study, investigators will use whole genome technologies to study c9orf72 in ALS tissue samples (including spinal cord and blood) to try to shed light on what the c9orf72 gene does and how the expanded repeat might cause ALS. Investigators will compare and contrast tissue samples from patients with an expanded repeat in c9orf72, sporadic ALS and control.

Researcher: Sanjay Kalra, M.D. University of Alberta; Edmonton, Alberta

Topic: Characterization of Cerebral Degeneration in ALS Using Texture Analysis.
**Funded by The EMD/ALS Biomarker Research Fund, in memory of Marsh Douthat, through The ALS Association, Keith Worthington Chapter*

Description: An accurate method to measure the extent of involvement of the brain in ALS currently does not exist. Such a measure would aid in earlier diagnosis, allowing for earlier start of treatment and access to clinical trials. Most importantly, it would assist in a more efficient means to evaluate new drugs, thus increasing the chances of finding an effective drug sooner as more compounds could be screened faster. This study incorporates state-of-the-art techniques in image processing methods with advanced magnetic resonance imaging (MRI) to measure different aspects of brain involvement in humans living with ALS.

Researcher: Greg Cox, Ph.D. Jackson Laboratories; Bar Harbor, Maine

Topic: Characterizing new models for ALS
**Funded by The ALS Association, Greater Philadelphia Chapter*

Description: The recent identification of pathways involved in RNA processing or RNA-mediated toxic species in familial and sporadic ALS provides a novel set of targets for therapy development. However, the specific cellular defects in motor neurons that render them susceptible to degeneration in the presence of abnormal RNA metabolism are currently unknown. The investigators propose to study disease mechanisms and RNA targets that make motor neurons susceptible to death using ES cell-derived motor neurons from two independent mouse models of neurodegeneration. The investigator's lab has generated unique reagents to facilitate these experiments from transgenic mice expressing a human ALS TDP43 (A315T) mutation and two novel mutant mouse strains that develop a severe and progressive motor neuron disease. The investigators found that a novel nuclear export-mediator factor (Nemf) and TDP43 proteins are mislocalized in motor neurons of their mutant mice, suggesting that NEMF regulates TDP43 localization. They hypothesize that altered nuclear/cytoplasmic trafficking of TDP43 and its RNA cargos underlies a cellular loss of function

mechanism in ALS motor neurons and that NEMF is critical for regulating TDP43 localization.

Researcher: Jeffrey Macklis, Ph.D., Harvard University; Cambridge, Massachusetts

Topic: Regeneration of Corticospinal Circuitry in ALS
** Funded by The ALS Association, Greater Philadelphia Chapter*

Description: Corticospinal motor neurons (CSMN) reside in cerebral cortex and connect to the spinal cord; their degeneration in ALS, along with degeneration of spinal motor neurons, causes spasticity and paralysis. CSMN are diverse, projecting to different levels in the cord (cervical, thoracic, and lumbar). ALS and related upper motor neuron disorders do not affect all CSMN equally; in bulbar forms of ALS, brainstem-projecting CSMN degenerate preferentially, while in hereditary spastic paraplegia (HSP), primarily lumbar-projecting CSMN degenerate. Hence, it will be optimal for future therapeutic strategies to generate appropriate, subtype-specific CSMN that project to appropriate levels of the spinal cord. The adult cerebral cortex has endogenous progenitor cells capable of generating new neurons. The investigator's lab identified genes that, in complex combination and sequence, control the development of CSMN at specific stages (from birth to final stages of axon extension and targeting). They propose to express a set of these rigorously selected molecular controls in specific adult progenitors to progressively build toward generating new CSMN in adult cortex. They will first direct them to differentiate into new neurons (these adult progenitor cells do not normally make neurons), direct them toward CSMN fates, and finally direct them to target selected levels in the spinal cord.

Researcher: Todd Golde, Ph.D., University of Florida; Gainsville, Florida

Topic: Neuroinflammation in ALS
**Funded by The ALS Association, Greater Philadelphia Chapter*

Description: Neuroinflammation has been implicated in aspects of ALS. Neuroinflammation is a general term used to define how immune cells of the brain and spinal cord respond to damage or infection. When they respond, these cells secrete a family of soluble proteins that act in concert to activate the immune system in much the same way that immune cells in other parts of the body would fight off an infection or respond to tissue damage. There is evidence that neuroinflammatory processes can both be harmful and protective. In studies of SOD1 mouse models of ALS, it has been shown that pro-inflammatory factors secreted by immune cells of the nervous system increase in levels at the time of disease onset. By contrast, the levels of ant-inflammatory factors are elevated early in disease course but decrease as disease progresses. Various therapeutic approaches have been used to manipulate the signalling pathways to respond to these cytokines, but there remain unanswered questions regarding the role of these cytokines in ALS pathogeneisis. The investigator's laboratory has had a long-standing interest in neuroinflammation in neurodegerative disease mainly Alzheimer's disease and has developed a number of novel tools to manuipulate these systems. They will now apply these tools to the SOD1 mouse model and propose to use a variety of recombinant adenoassocated virus vectors that

express various neuroinflammatory modulators to rigorously test the role of inflammatory cytokines in ALS disease pathogenesis.

Researcher: Pavel Ivanov, Ph.D., Brigham and Womens Hospital; Boston, Massachusetts

Topic: Angiogenin and amyotrophic lateral sclerosis

Description: Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disorder of devastating impact that causes selective injury and loss of motor neurons. Both familial and sporadic ALS forms are phenotypically indistinguishable from each other suggesting the existence of common pathways underlying disease pathogenesis. The recent identification of ALS-associated mutations in several RNA-processing proteins implicates aberrant RNA metabolism as a common basis of neuronal death. The ribonuclease angiogenin (ANG) is one of the RNA-processing proteins in which loss-of-function mutations are found in patients with both familial and sporadic ALS, although ANG-dependent changes in cell physiology contributing to the development of ALS are not known. Significantly, treatment of motor neurons with recombinant wild type ANG, but not its ALS-associated mutants, protects them from stress-induced death and apoptosis. Mechanisms of ANG-mediated neuroprotection are currently unknown, but the ribonuclease activity of ANG is an absolute requirement for the cytoprotection. Investigators showed that ANG is a stress-responsive factor that cleaves tRNA to produce a new class of small RNAs, tRNA-derived stress-induced RNAs (tiRNAs). In their proposed study, investigators plan to characterize the roles of ANG and tiRNAs in cell physiology and to examine the hypothesis that ANG-mediated changes in RNA metabolism contribute to the pathophysiology of ALS.

Researcher: Randall Tibbetts, Ph.D. University of Wisconsin, Madison, Wisconsin

Topic: Proteostatic regulation of ubiquilins in ALS
**Funded by The ALS Association, Wisconsin Chapter*

Description: The investigator's goal is to understand how mutations in two closely related genes, Ubiquilin 1 (UBQLN1) and Ubiquilin 2 (UBQLN2), contribute to neurodegeneration in Alzheimer's Disease (AD) and ALS, respectively. Ubiquilins play important roles in the clearance of misfolded and/or aggregated proteins from neurons. Previous studies showed that a mutation in UBQLN1 was associated with increased risk for AD, and work from the investigators laboratory has provided novel insights into how the AD-associated UBQLN1 mutation, termed UBQLN18i, alters UBQLN1-mediated protein clearance. Additionally, they found that UBQLN1 contributes to the clearance of TDP-43, an aggregation-prone RNA-binding protein that is deeply implicated in the neuropathogenesis of ALS. UBQLN1 promotes degradation of TDP-43 by autophagy, a conserved process that mediates destruction of toxic protein aggregates. The recent finding that mutations in the related *UBQLN2* gene cause ALS further supports a crucial role for the Ubiquilin family in suppressing ALS, and the investigators have begun to probe the biochemical consequences of ALS-associated UBQLN2 mutations. As part of these studies, they will generate mouse models to study the impacts of disrupting UBQLN1 and UBQLN2 function on the nervous system. These models

will provide new insights into the pathogenesis of ALS caused by deregulation of the Ubiquilin pathway.

Researcher: Jiou Wang, Ph.D. Johns Hopkins University, Baltimore, Maryland

Topic: Identifying disease pathways and therapeutic targets in novel models of ALS
**Funded by Corinne Schwartz and Family*

Description: Amyotrophic lateral sclerosis (ALS) is an age-dependent neurodegenerative disease that is characterized by the degeneration of motor neurons. Discoveries of gene mutations linked to ALS have accelerated our understanding of the molecular underpinning of this devastating disease. Recently, the expansion of a hexanucleotide repeat (HNR) in the *C9orf72* gene was found to cause ALS. It remains a mystery how this genetic abnormality brings about the development of ALS. Investigators recently demonstrated that a simple nematode (round worm), *Caenorhabditis elegans,* could be genetically engineered to express ALS genes and exhibit disease-like phenotypes. The short-lived, transparent *C. elegans* is an ideal paradigm for dissecting the complex disease processes. The investigators plan to use the *C. elegans* models to conduct unbiased screens to identify modifiers that influence the age-dependent phenotypes induced by the toxicity of the abnormal HNR. Identification and characterization of the toxicity modifiers in the *C. elegans* systems may lead to promising drug targets that can be extended to mammalian systems and then quickly developed to combat ALS.

Researcher: Wim Robberecht, M.D., Vesalius Research Center; Leuven, Belgium

Topic: *The Ephrin Axon Repellent System in ALS*

Description: In previous work using a zebrafish model, investigators have found that the molecule EphA4 is a modifier of amyotrophic lateral sclerosis (ALS). EphA4 is a receptor in a nerve guidance pathway, the ephrin system, which guides nerves during development by repelling them from certain targets. The role of this system in neurodegenerative disease is almost unexplored. Investigators found that genetic deletion of EphA4 rescues the ALS phenotype in the zebrafish model and increases survival in the ALS mouse model and rescues its motor neurons and neuromuscular junctions. Blockade of the receptor with drugs rescues the zebrafish phenotype and increases survival in the ALS rat. In addition, high expression of this receptor in patients with ALS is associated with more aggressive disease. In the current project, investigators intend to elucidate the mechanism through which EphA4 affects motor neuron degeneration. They will study the signaling mechanism of the EphA4 receptor in ALS and investigate which factor stimulates this receptor during progression of the disease. Furthermore, they will investigate whether a drug that interferes with the intracellular cascade initiated by EphA4 stimulation can affect ALS. This is of interest as this cascade is used by a variety of other nerve outgrowth inhibitory systems.

Researcher: Brian McCabe, Ph.D. Columbia University, New York, New York

Topic: Disruption of neuronal NF-kB signaling by TDP43 and FUS/TLS mutations in familial ALS

** Funded by The ALS Association, Greater New York Chapter*

Description: FUS/TLS and TDP-43 are two genes linked to both sporadic and familial ALS (fALS) in addition to frontotemporal dementia. In recent studies, investigators have shown these proteins work together in a common molecular pathway in neurons. In this proposal investigators will investigate the ALS relevant molecular and cellular consequences of this interaction. Their preliminary data suggest that NF-kB signaling, an important evolutionarily conserved molecular pathway which has been genetically linked to ALS, could be disrupted by loss of FUS and TDP-43. If this hypothesis is proven correct, existing compounds that modulate this pathway could be investigated as potential treatments for ALS.

Researcher: Yimin Zou, Ph.D. University of California; San Diego, California

Topic: Characterizing the role of Wnt Signaling in motor neuron survival and ALS Models
**Funded by the California Chapters of The ALS Association*

Description: The neurons that control muscle movement are called motor neurons. After motor neurons mature, they live in our bodies throughout our lives. When they die, such as in ALS, patients lose the ability to move. Both the motor neurons in our brain and those in our spinal cord have long axons, which project from our cortex to our spinal cord and from our spinal cord to our muscles, respectively. Maintaining these axons is not a trivial task, and these axons are vulnerable to injury or toxicity, which can cause axon degeneration. Motor axon degeneration occurs before the death of the motor neuron cell bodies themselves. Understanding how neurons normally maintain the survival of their axons may allow us to understand how they degenerate and provide new strategies to stop or slow down degeneration and thus develop new treatments of ALS. We found that a component signaling system, called “Wnt signaling,” is a very potent regulator of motor axon survival. In the well-known animal model of ALS, SOD1 mutant mice, Wnt signaling becomes disregulated. The investigators propose to directly test whether this change of Wnt signaling causes ALS. If correct, they may have discovered a new approach to treat ALS.

Researchers: Guy Rouleau, M.D., Ph.D., CHU Ste Justine Research Center; Montreal, Quebec, Canada; and Christopher Person, Ph.D., The Hospital for Sick Children; Toronto, Ontario, Canada

Topic: Characterization of C9orf72 expansion stability in ALS/ FTD patients
**Funded by The Robert Abendroth Genetics Fund*

Description: Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD) are two diseases that can occur separately or together in families and individuals. Very recently, a large repeat expansion (GGGGCC) in the *C9orf72* gene has been identified as the most common cause of familial and sporadic ALS and FTD. Numerous neurodegenerative/muscular diseases are due to similar expansions of DNA repeat sequences that alter the production of proteins, leading to toxic products. Investigators propose to measure the size of the *C9orf72* repeat in 1,500 individuals and examine the transmission from parents to offspring to assess the stability of this repeated portion of the gene during parent to offspring

transmission and between neuronal and non-neuronal tissues. They are also going to test if any instability is due to genetic variations in genes encoding DNA repair genes. Identifying such mechanisms may help to better understand the disease and lead to novel therapeutic avenues.

Researcher: Clive Svendsen, Ph.D. Cedar-Sinai Medical Center; Los Angeles, California

Topic: Combining muscle-derived GDNF delivery and spinal stem cell transplants to provide motor neuron survival and function in an ALS rat model
**Funded by The Neil Brourman, M.D., ALS Research Fund*

Description: Therapeutic strategies for effective treatment in ALS will likely require the combination of several therapeutic approaches. Using various approaches, the first goal of this study is to confirm the overt neuroprotective effect of glial cell line-derived neurotrophic factor (GDNF) on motor neurons survival and function in the SOD1G93A rat model of ALS. Secondly, the investigators propose combining the viral delivery of the growth factor glial cell line-derived neurotrophic factor (GDNF) to the muscles of SOD1G93A rats with the spinal transplantation of human neural progenitors/stem cells (hNPCs) secreting GDNF. Their hope is to generate a combination of treatments with significant beneficial additive effects that can be translated to clinical trials. Moreover, the identification of a successful combination will lead to further studies by this group to identify strategies that would further enhance the therapeutic effect of our approach.

Researcher: Heather Durham, Ph.D. Montreal Neurological Institute; Montreal, Quebec, Canada

Topic: Involvement of Protein Arginine Methyl Transferase (PRMT1) in ALS6

Description: Amyotrophic lateral sclerosis (ALS, Lou Gehrig's Disease) is a fatal neurodegenerative disorder characterized by the loss of certain neurons that relay messages from the brain to skeletal muscles. The result is gradual loss of the ability to move, to swallow, to speak and eventually to breathe, but most other bodily functions remain intact. Although the cause of most cases of ALS is not known, genetic mutations have been identified in forms that run in families. One such form, ALS6, is caused by mutations in FUS, a constituent of protein complexes that transport RNA from the nucleus throughout the cell where it serves as the template for synthesis of proteins where they are needed. In neurons, the distribution of these RNA containing complexes is particularly important for maintaining synaptic connections with other neurons and for responding to the level of neuronal activity and stress. ALS-causing FUS mutants are mislocalized in the cell, indicating that trafficking of not only FUS, but its partners and RNA could be compromised. Methylation is a modification of these proteins that is important for their localization. Investigators found that PRMT1, an enzyme required for methylation, follows FUS and accumulates with mutant FUS in the cytoplasm of cultured motor neurons. The investigators' research will address how depletion of PRMT1 from the nucleus affects its many important functions including adapting gene expression to environmental signals and will determine if mutant FUS disrupts the localization and function of other interacting proteins and RNA. They will also determine if mislocalization of PRMT1 and

FUS-interacting proteins also occurs in sporadic and other familial forms of ALS, and thus has relevance beyond ALS6.

Research: Christine Beattie, Ph.D. Ohio State University; Columbus, Ohio

Topic: Identifying the initial mechanisms of nervous system dysfunction in ALS

Description: Current ALS research has not translated to successful treatments in patients. New approaches are needed, especially in the search of effective drug compounds. Zebrafish offer many strengths in terms of similar genetics and neuroanatomy when compared to mammals, large numbers, small size, and ease of drug testing. Investigators are using this model to look for early changes in the spinal cord that begin a cascade of events leading to the eventual dysfunction of motoneurons. Their hypothesis is that mutated SOD1 increases motoneuron excitability by altering AMPA receptors leading to a disruption of calcium signaling between the ER-mitochondria that are important to maintain motoneuron health. Stress at the ER causes an initially beneficial condition called the unfolded protein response (UPR); however, when the cell is overstressed the UPR becomes damaging. They are testing their hypothesis using a combination of electrophysiology, calcium imaging, and RNA expression analysis. The knowledge they gain from these experiments will be used to not only understand the early changes that happen in motoneurons in this disease but will also define biomarkers that in future work will aid in the design of high throughput screens in zebrafish larvae to aid in drug development and testing.

MILTON SAFENOWITZ POST DOCTORAL FELLOWSHIPS:
The ALS Association offers two-year Milton Safenowitz Post-Doctoral Fellowships. This award is made possible by the generosity of the Safenowitz family through the Greater New York Chapter of The ALS Association and is in memory of Mr. Safenowitz, who died of ALS in 1998. These awards are for young, recently graduated scientists, in an effort to attract them to enter the ALS field and stimulate cutting-edge scientific discovery. Funding is also provided by the generosity of Edmund McCurtain.

Researcher: Helene Tran, Ph.D., University of Massachusetts Medical School; Worcester, Massachusetts

Topic: A drosophila model of the hexanucleotide repeats-induced toxicity in ALS

Description: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by progressive loss of motor neurons. Unfortunately, there is no effective treatment. The etiology of ALS is complex, combining both environmental and genetic factors. Recently, two independent studies identified an abnormal (GGGGCC)n repeat expansion in a noncoding region of the *C9ORF72* gene in many cases of sporadic and familial ALS. To determine whether this repeat expansion contributes to the physiopathology of ALS and to uncover the underlying pathogenic pathways, the investigator proposes to develop a novel model organism expressing these repeats. The fruit fly is a powerful tool to elucidate these questions. Fundamental cellular processes related to neurobiology are highly conserved from flies to humans. In addition, flies have numerous advantages as an experimental model, including the ease of genetic manipulations, rapid propagation, and short life spans. Therefore, establishing

transgenic flies expressing this ALS/FTD-associated (GGGGCC)n repeat expansion as a novel organism model of ALS will rapidly bring new insights into mechanistic questions and provide a valuable tool for evaluating prospective therapeutic strategies.

Researcher: Jacob Ayers, Ph.D., University of Florida; Gainesville, Florida

Topic: Pathogenic role of wild-type superoxide dismutase in ALS

Description: Mutations in superoxide dismutase 1 (SOD1) account for a major fraction of the inherited forms of ALS, and several studies have implicated its role in sporadic forms of the disease, which account for approximately 90% of all ALS cases. In this proposal, investigators aim to investigate the potential for normally folded, wild-type superoxide dismutase 1 (SOD1) to adopt characteristics similar to mutant versions of the protein, including misfolding, formation of protein inclusions and toxicity. They intend to use various techniques to first induce a focal accumulation of mutant SOD1 in the spinal cord and then determine its effects on the properties of wild-type SOD1 and whether these effects can propagate throughout the central nervous system (CNS). Additionally, the investigators plan to study whether these changes in properties may occur through direct interactions between the two proteins and whether they are influenced by an inflammatory response within the CNS. The potential neurotoxic mechanisms examined in this proposal will not only be paramount in understanding the contributions of SOD1 in sporadic forms of ALS but may also have important therapeutic implications.

Researcher: Regina Maria Kolaitis, Ph.D., St Jude Children's Research Hospital; Memphis, Tennessee

Topic: The role of VCP in regulating the assembly and disassembly of stress granules

Description: Perturbed RNA metabolism is likely a major contributor to ALS pathogenesis. RNA-binding proteins (RBPs) are a major component of the pathological inclusions in most sporadic and familial ALS patients. Moreover, mutations in RBPs, TDP-43 and FUS/TLS, account for a small number of ALS cases. This study focuses on VCP, a protein found in the pathological inclusions of ALS. Mutations in VCP cause a multisystem TDP-43-opathy that includes motor neuron disease. Moreover, VCP mutations account for a small percentage of fALS cases. The investigators have recently discovered a role for VCP in regulating RNA metabolism, and their evidence suggests that this role of VCP is compromised by disease-causing mutations. Prior members of the lab discovered genetic interaction between VCP and TDP-43 (and other RBPs). Subsequently, the investigators discovered physical interaction between VCP and TDP-43 (and other RBPs). Upon joining the lab, Dr. Kolaitis set out to elucidate the relationship between VCP and RBPs (including TDP-43 and FUS). Over the past 9 months, she has learned that VCP plays an essential role in the dynamics of a cytoplasmic ribonucleoprotein particle called a stress granule (SG). SGs contain repressed translation complexes and form in response to a variety of stressors. SGs represent posttranscriptional "reprogramming" – a reversible mechanism whereby the cell can limit translation of non-essential mRNAs under stress conditions without permanently eliminating these

transcripts. Upon restoration of normal conditions, SGs are disassembled and the repressed mRNAs are returned to the translational pool. TDP-43 and FUS/TLS (and presumably their target mRNAs) are recruited to SGs, and this is enhanced by ALS-causing mutations. The investigator has established a dynamic imaging system that permits monitoring of SG assembly and disassembly. In preliminary studies, she has shown that VCP is recruited to SGs and is essential for SG disassembly, including the liberation of TDP-43 from this structure. Moreover, ALS-causing mutations in VCP lead to impaired SG disassembly. This proposal is designed to test the hypothesis that VCP regulates the dynamics of SG disassembly, and this is the point of intersection with TDP-43 and FUS in ALS pathogenesis.

Researcher: Brandi-Davis Dusenbery, Ph.D. Harvard University, Stem Cell and Regenerative Medicine; Cambridge, Massachusetts

Topic: Disrupted micro RNA biogenesis in ALS patient derived motor neurons

Description: microRNAs are a recently discovered class of regulatory molecules that control diverse processes, including cell death. In particular, miRNAs are essential for the survival of motor neurons (MNs), the cell type which is lost in ALS. Interestingly, the protein machinery that makes microRNAs was found to associate with two proteins known to be disrupted in ALS, TDP-43 and FUS. This suggests that disruption of TDP-43/FUS may lead to alteration in microRNA production, which may in turn lead to neurodegeneration. TDP-43 and FUS are expressed in many different cell types; however, MNs are selectively affected by their disruption. Thus, it is critical to study the function of TDP-43/FUS in MNs. Although several mouse models have been developed to study ALS, the results of these studies have failed to provide a significant clinical advance, perhaps because the underlying mechanism of MN loss in ALS is specific to human cells. The use of induced pluripotent stem cell (iPSC) technology allows skin cells from ALS patients to be converted into pluripotent cells, which can then be differentiated into large numbers of human MNs. The investigator proposes to use these cells to probe how TDP-43/FUS regulate microRNA biogenesis and how their disruption leads to motor neuron disease. As microRNAs represent direct drugable targets, the identification and characterization of miRNAs disrupted in ALS could provide novel therapeutic opportunities. Moreover, these studies may allow the identification of a miRNA signature of ALS that could be utilized as biomarkers for diagnostic purposes.

Researcher: Dimitry Yudin, Ph.D. Columbia University Medical Center; New York, New York

Topic: Contribution of neurotensin to degeneration of vulnerable motor neurons in ALS

Description: Even in late-stage ALS patients, certain motor functions such as movement of the eyes and continence are preserved. This phenomenon is explained by the striking resistance to degeneration of the motor neurons that drive the corresponding muscles. If we could confer even part of that resistance on the motor neurons in the spinal cord that normally degenerate in ALS patients, we would have identified a strong new therapeutic strategy. One way of doing this is to identify genes that are selectively expressed in vulnerable spinal motor

neurons and then to inactivate them either genetically or pharmacologically, starting in mouse models of ALS. The investigators recently showed that one gene expressed in vulnerable motor neurons is neurotensin, a 13-amino acid peptide whose role has never been studied in the neuromuscular system. However, it has multiple properties that suggest it might contribute to neurodegeneration. Neurotensin reinforces excitotoxicity in models of Parkinson's disease, contributes to neurodegeneration in stroke, and activates inflammatory processes in the brain. The investigator, therefore, proposes that neurotensin may contribute to motor neuron degeneration and neuroinflammation in ALS. He will test this first by studying the precise subset of motor neurons in which neurotensin and its receptors are expressed. Next, he will ask whether inactivation of neurotensin in mutant SOD1 mice, the most frequently studied model of ALS, delays muscle paralysis and prolongs lifespan. Lastly, he will ask whether neurotensin makes mouse and human motor neurons more vulnerable to disease-related stressors. Thus this study should uncover new details of the disease mechanism in ALS and hopefully will help identify a new therapeutic target in this currently incurable disease.

THE ALS ASSOCIATION-INITIATED AWARDS

Investigators submit proposals in response to topics The ALS Association and its advisors have determined are priority areas of research. The ALS Association convenes a review board to discuss proposals and makes selections based on the merits of each.

Researchers: Brian K. Kaspar, Ph.D., The Research Institute at Nationwide Children's Hospital, Columbus, Ohio; David V. Schaffer, Ph.D. University of California-Berkeley, Berkeley, California; and Dwight E. Bergles, Ph.D., Johns Hopkins University, Baltimore, Maryland

Topic: Development of Gene Delivery to Oligodendrocytes
* *Funded in Partnership with P2ALS*

Description: Inherited forms of amyotrophic lateral sclerosis (ALS) have been linked to mutations in genes that are widely expressed by both neurons and glial cells. In mouse models of ALS, deletion of mutant genes from glial cells can significantly prolong life, indicating that these non-neuronal cells are important contributors to this disease. Our recent studies in G93A SOD1 mice show that oligodendrocytes, a class of glia that form myelin sheaths around axons, degenerate in the spinal cord and that selective removal of mutant SOD1 from oligodendrocytes prolongs life. These studies indicate that oligodendrocytes are an important target for therapeutic manipulation in vivo. Although it is possible to manipulate gene expression in oligodendrocytes in mice using complex transgenic manipulation, such approaches are not applicable for therapeutic intervention in humans. We propose to use a novel technique known as molecular evolution to engineer an adeno-associated viral vector (AAV) that will allow high efficiency infection of ligodendrocytes. Generation of this vector will help define the mechanisms responsible for oligodendrocyte degeneration in ALS and enable development of new strategies to minimize the toxic effects of these mutant proteins in vivo.

Researcher: Serge Przedborski, M.D., Columbia University; New York, New York

Topic: Cell-based assay for the screening of neuroprotective small molecules for ALS

**Funded by The ALS Association, Greater New York Chapter*

Description: Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's Disease in the USA, is a common fatal paralytic disorder affecting adults. Thus far, there is no treatment for this debilitating disease. The team involved in this project is composed of researchers from both Columbia and Harvard Universities who have identified and validated the means to reproduce ALS in a laboratory dish. These advances have not only shed light onto the understanding of how brain cells die in ALS but have also led to the development of cell models of ALS that can be used within new technologies for the screening of a large number of small molecules for protective compounds. Compared to currently available cell models, these cell systems have two potential advantages for drug screening: they show spontaneous death and use cells that can be readily expanded and that can be coaxed into the exact same brain cell type that the one which die in ALS. Herein, it is proposed to use this model of ALS to perform large-scale drug screening. The investigators are confident that the comprehensive set of investigations proposed in this study can play a decisive role in identifying small molecules with protective properties that are directly relevant to ALS.

For questions on these and other research grants, please email: researchgrants@alsa-national.org.