

# RESEARCH ALS TODAY

THE ALS ASSOCIATION | VOLUME 7 | FALL 2010

## Rodent Models of TDP-43 Proteinopathy by Leonard Petrucelli, Ph.D. Mayo Clinic, Jacksonville, Fla.

**Thanks to the recent creation** of genetically engineered mice and rats, investigators have uncovered new clues about how Tar DNA-binding protein (TDP-43) may contribute to ALS. The presence of inclusions (abnormal clumps of proteins) within neurons is a pathological hallmark of ALS. Four years ago, TDP-43 was identified as a major component of these inclusions in most ALS cases. Soon thereafter, mutations in the gene encoding TDP-43 were linked to ALS, confirming that TDP-43 is directly involved in disease pathogenesis. In order to decipher how TDP-43 contributes to the development and progression of ALS, at least seven research groups have generated transgenic TDP-43 rodent models [1-7].

To determine how a protein runs amuck in disease, it is ideal to first have a good understanding of how that protein behaves under normal circumstances. Usually, TDP-43 takes residence in the nucleus of neurons. In ALS, however, TDP-43 accumulates in the cytoplasm, where it forms the aforementioned inclusions. Furthermore, TDP-43 undergoes several modifications in ALS that are not normally observed; it becomes ubiquitinated, phosphorylated and cleaved to generate small TDP-43 fragments. At present, little is known regarding how these changes in TDP-43



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lead to disease. Nonetheless, the gaps in our knowledge will surely narrow now that animal models of TDP-43 are available.

Many commonalities exist among the various rodent models engineered to express high levels of TDP-43, despite the fact that TDP-43 may have been expressed in different forms (i.e. wild-type vs. mutant) or in different brain regions [1-7]. For instance, motor function impairments, increased ubiquitin, TDP-43 fragmentation, gliosis, axonal degeneration, neuronal loss, and shortened lifespan were observed in most transgenic animals (Table 1). The findings generated from these models indicate that both wild-type and mutant TDP-43 are toxic. Also discovered, to the

surprise of investigators, was that many of the transgenic animals displayed neuronal ubiquitin inclusions, but TDP-43 was not always a component of these inclusions. While this suggests that TDP-43 neurotoxicity can occur even in the absence of TDP-43 inclusions, it does not exclude their pathogenic potential. As with other neurodegenerative diseases, like Alzheimer's and Parkinson's diseases, it may be difficult to resolve if protein aggregates are themselves toxic, merely an inert by-product, or a protective mechanism used by cells to sequester harmful protein species.

As seen in ALS, small TDP-43 fragments were frequently observed in mice and rats

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## Exciting New Advances in Technology Enable Progress in ALS Research

**This edition highlights the advances made** in understanding the underlying mechanisms involved in ALS. Significant progress has been made to develop new model systems since the recent discovery of TDP-43 and FUS-TLS linked to familial ALS, as described by investigators at Mayo Clinic, Jacksonville, Florida. There is still a great deal to learn about the biology of these proteins and their role in cells and, more specifically, how mutations can lead to ALS. The model systems will be important tools to begin to unravel these mechanisms. Rodent models will be complemented by the study of motor neurons and glia generated from human skin cells as described by investigators from Columbia. Using novel technology, investigators can determine the molecular profile of these cells and start to dissect differences between cells derived from healthy individuals and those with ALS. Furthermore, they will be able to develop model systems in a dish, which can be more readily manipulated to develop novel therapies.



Lucie Bruijn, Ph.D.  
Chief Scientist  
The ALS Association

The ALS Association encourages young investigators to enter into the field of ALS through the Milton Safenowitz Post-Doctoral Fellowship Program and work from these investigators is featured throughout this edition of *Research ALS Today*. Dr. Da Cruz's studies have looked carefully at the role of mitochondria in ALS, important organelles supplying energy to the cells. Understanding how they are involved in the disease process will provide clues for therapeutic intervention.

This is a tremendously exciting time for investigators to become involved in ALS research. Through increased collaborations, resource sharing and amazing advances in technology, we could not be better positioned to tackle the difficult questions in understanding ALS disease with a goal to develop therapies to alter the course of the disease.

- Lucie Bruijn, Ph.D.

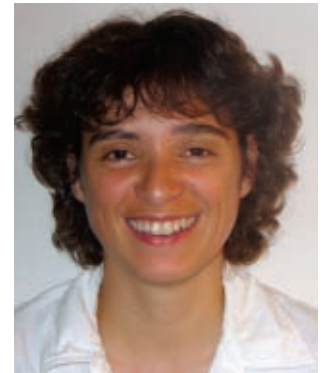
## Blame It on the Power House

by Sandrine Da Cruz, Ph.D., UCSD  
Milton Safenowitz Post-Doctoral Fellow

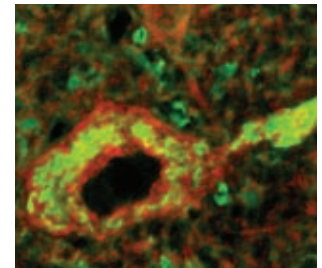
In the past years, major advances in understanding the mechanisms underlying ALS pathogenesis have been made thanks to the use of mouse models, stem cells and genetics. Although it is still unclear what causes the retraction of motor neurons from the muscle, ultimately leading to their premature death in ALS, evidence from many experimental directions supports the proposal that ALS pathogenesis is not due to a unique toxic event but rather a convergence of damage within multiple cells.

One of the central features of ALS that has emerged in recent years is damage to mitochondria, the intracellular organelles that consume oxygen to produce the chemical fuel that powers all cell functions. Indeed, abnormal mitochondrial morphology, deficits in the ability of mitochondria to produce chemical energy and damage to those mitochondria so that they produce toxic forms of oxygen have been reported in ALS patients and mouse models that are genetic mimics of an inherited form of ALS. Yet it is a matter of debate whether mitochondrial dysfunctions are a cause or a consequence of neuronal degeneration during ALS, and if the latter, whether mitochondrial damage is a downstream contributor to pathogenesis. Answering this question is critical not only to further our understanding of the basic mechanisms underlying ALS pathogenesis, but more important it will determine whether therapies that modulate mitochondrial function represent a viable approach to treat ALS.

Mitochondria are organelles that play a crucial role in several cellular processes within a cell. They are the primary site of



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Presence of misfolded SOD1 (green) in mitochondria (red) of a spinal cord motor neuron from symptomatic mice expressing an ALS linked mutant SOD1.

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## Power House

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energy production and are essential for maintaining ion homeostasis, in particular for calcium. However, mitochondria are also the main source of reactive oxygen species and are key players in apoptotic cell death. Given the central role played by mitochondria, it is not surprising that when they do not function properly this is deleterious to most cells, in particular neurons, which have high energy demands and, are subject to large amplitude ionic fluxes.

The first indication that mitochondria may be a target for toxicity in ALS came from electron microscopy studies reporting abnormal mitochondrial morphology in the motor neurons and muscle cells of both familial and sporadic ALS patients, as well as in mouse models of an inherited form of ALS that express mutants of SOD1. Of note, alteration in the morphology of mitochondria is also evident in neurons from mice expressing human TDP-43, a gene recently linked to both sporadic and familial forms of ALS. A number of studies have also reported a range of defects in oxidative phosphorylation, respiratory complex activity, membrane potential, mitochondrial genome, calcium buffering capacity and energy production of mitochondria isolated from spinal cords, brains and/or muscles of biopsied patients and/or mutant SOD1 transgenic mice.

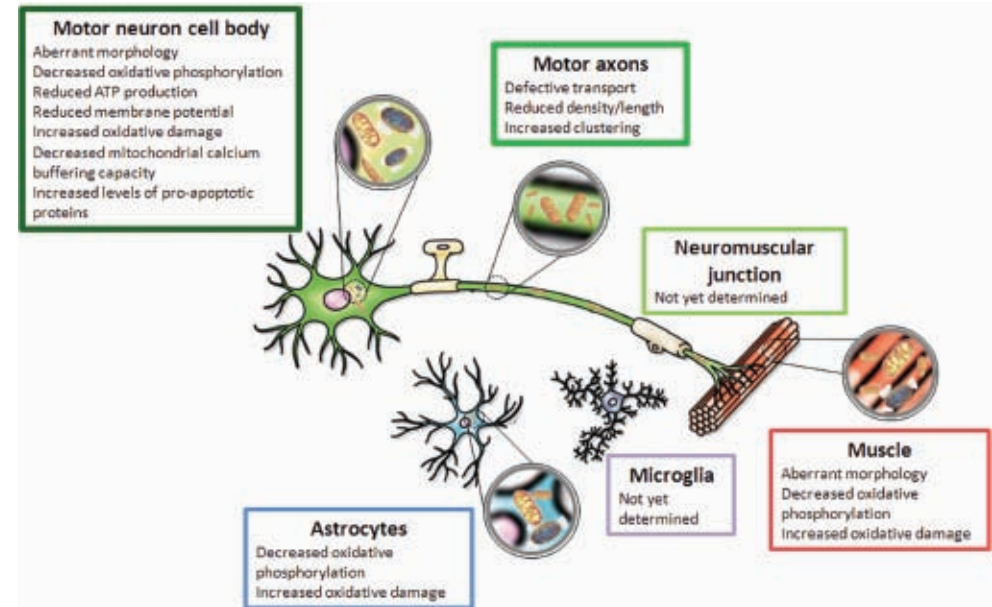
In addition, evidence obtained both in cell culture and rodent models of ALS supports the proposal that the transport and dynamics of mitochondria along the axons of motor neurons is disrupted by expression of mutant SOD1. However, it is important to mention that the mitochondrial dysfunctions outlined here have not consistently and systematically been associated with all the human ALS cases

or the different types of mutant SOD1 mouse models.

More direct evidence suggesting that mitochondria are contributors to ALS pathogenesis comes from several reports showing that mutant SOD1, which is predominantly found in the cytosol of healthy cells, preferentially associates with mitochondria extracted from tissues that are affected during ALS pathogenesis but not from unaffected tissues. This association is thought to cause some, if not all, the mitochondrial dysfunctions described above which ultimately could trigger the death of motor neurons in ALS. This year, two studies have provided evidence supporting this hypothesis. Pedrini and coworkers propose that mutant SOD1 through its association at the mitochondria with Bcl-2 induces changes in mitochondrial morphology, release of cytochrome c and a reduction in cell viability due to the conformational conversion of the established anti-apoptotic function of Bcl-2 into a toxic protein species. Israelson and colleagues revealed a different target of mutant SOD1 at the mitochondria. The authors showed that mutant SOD1 interacts with voltage-dependent anion channel-1 (VDAC1), a key outer membrane mitochondrial component that plays an essential role in ATP/ADP transport, calcium homeostasis and cell death. Through this direct interaction, mutant SOD1 not only inhibits the conductance of VDAC1 in a reconstituted lipid bilayer but also leads to reduced ADP accumulation in spinal cord mitochondria of mutant SOD1 mouse model. Furthermore, reduction of VDAC1 activity by targeted gene disruption significantly reduces the lifespan of mutant SOD1 transgenic mice.

Altogether these studies propose that mutant SOD1, by binding to at least two mitochondrial partners, causes a wide

## Mitochondrial dysfunctions in cell types most affected during ALS pathogenesis



Summary of the mitochondrial dysfunctions that have been reported in the different cell types in ALS patients and/or ALS rodent models.

range of mitochondrial dysfunctions which may lead to the death of the motor neurons. Alterations in mitochondrial function have mostly been observed using biochemical methods, which do not allow distinguishing in which specific cell type(s) the dysfunctional mitochondria are found. Since it is now established that damage within motor neurons is enhanced by damage incurred by nonneuronal neighboring cells (in particular astrocytes and microglia), it is likely that mitochondrial defects occur not only in motor neurons but also in the surrounding cells.

Interestingly astrocytes expressing mutant SOD1 have been reported to induce mitochondrial defects within motor neurons (Bilsland, 2008) and damage to the mitochondria within astrocytes from mutant SOD1 transgenic rats causes acute motor neuron death in astrocyte-motor neuron cocultures (Cassina, 2008). Altogether it

will be of interest to investigate the role that mitochondria from the cells neighboring the motor neurons play in ALS pathogenesis.

The discovery that mitochondria are damaged in ALS pathogenesis has served as a basis for the testing in clinical trials of compounds that have been proposed to improve mitochondrial function either directly or indirectly. Some of these drugs, such as the mitochondrial antioxidant coenzyme Q or the permeability transition pore inhibitors MCI-186 and TRO19622, are currently being tested in Phase II trials.

Based on the experimental data available at present it is clear that mitochondria are damaged in ALS patients and ALS rodent models. It is now essential to test whether mitochondrial damage is a primary contributor to death of motor neurons, and possibly other cells in ALS pathogenesis. In the

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# Recent Efforts to Understand ALS Disease Mechanisms



For more than 150 years, clinicians and scientists have known that ALS is a debilitating disease that causes progressive loss of motor function through the death of neurons that control movement. Only recently, however, has it been possible to investigate the mechanisms underlying this currently incurable disease. Technological and conceptual advances in neuroscience, biochemistry and genetics, among other disciplines, have made it possible to begin to tease out an understanding of this complex disorder. In the past, our laboratory has studied the basic mechanisms of cellular functions, but recently we have turned our attention to ALS. Our goal is to use our understanding of molecular and cellular biology and cutting-edge technology to investigate the causes of ALS. Our hope is that this approach will provide the information necessary to develop drugs to treat ALS.

We have taken a multifaceted approach to the study of ALS disease mechanisms. We use mouse models of ALS to study the interactions between motor neurons (the cells of the nervous system that control movement) and glia, (the cells that support and surround neurons in the spinal cord). With the advent of the new stem cell technologies, we have also initiated studies aimed at exploring the interactions between neurons and glia in human ALS. In both cases we use the latest tools developed to study basic cellular mechanisms.

In both the mouse and human studies we rely heavily on the use of stem cells, which

have the potential of becoming any cell type (pluripotent) including motor neurons and glia. Because neurons are terminally differentiated cells, i.e. they cannot divide to make more of themselves, it is difficult to obtain enough cells to study, and it is difficult to maintain them in culture dishes for the duration of our experiments. Therefore, instead of using neurons that we obtain directly from mice or humans, we study motor neurons produced from stem cells.

In this case the cells multiply to large numbers in culture before being turned into motor neurons or glia. Because of the ethical issues surrounding the use of human stem cells, our initial studies focused on mouse models of ALS.

However, recently a new way of obtaining stem cells from humans revolutionized the field of stem cell biology and provided us with a way to produce motor neurons from skin cells obtained from ALS patients! A description of both of our mouse model and human studies follows.

In the first approach, we collaborated with the laboratory of Dr. Kevin Eggan to make use of a well-established mouse model of ALS in which a human gene called SOD1 was inserted into the mouse genome. Mutations in the SOD1 gene have been shown to cause one type of familial ALS. Remarkably, the mice carrying human SOD1 genes with the mutation that causes ALS exhibit motor defects comparable to those seen in ALS patients. Using stem

cells from the SOD1 ALS mice model we generate motor neurons in culture dishes that we can then study and characterize. At the cellular level these motor neurons exhibit properties of ALS that are found in motor neurons in humans and mice with the disease.

We were also able to study interactions between the ES cell-derived motor neurons and glia in a culture dish. We study all combinations of normal and ALS motor neurons and glia, and find that glia bearing the SOD1 mutation that causes ALS release factors that are toxic to both normal and ALS neurons. We are very grateful to The ALS Association for supporting this research.

We are currently in the second phase of these studies in which we use revolutionary high-throughput gene expression tools to study the inter-actions between ALS and non-ALS neurons and glia. The objective of this research is to identify genes or pathways that cause ALS when altered, and to identify the toxic factors released by glia.

Because this research was performed in mice based on a mutation that is fairly rare in human ALS, we wanted to move to a human model that will allow us to study other familial forms of ALS and sporadic ALS. Fortunately, Dr. Shinya Yamanaka from The University of Kyoto developed a ground-breaking technology

by Monica Carrasco, Ph.D. and Tom Maniatis, Ph.D.



Monica Carrasco, Ph.D.  
Milton Safenowitz  
Post-Doctoral Fellow



Tom Maniatis, Ph.D.  
Columbia University, N.Y.

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*“...instead of using neurons that we obtain directly from mice or humans, we study motor neurons produced from stem cells.”*

## timeline

1840	1850	1860	1870	1880	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990				
		1869: French neurologist Jean-Martin Charcot identifies ALS									50s: DNA structure solved	50s: Nerve growth factor (NGF) identified—protective, growth promoting factor for nerve cells	1968: SOD1 enzyme identified	70s: Programmed cell death in motor neurons demonstrated	1985: The ALS Association funds study of inherited motor neuron disease	1986: Genes for muscular dystrophy identified	1989: The ALS Association funds search for a common genetic link to ALS	1990: Congress declares the 1990s the “Decade of the Brain”	1990: Growth factor CNTF is found to increase survival of motor neurons

## ALS Disease Mechanisms

Continued from page 4

for the re-programming of human skin cells to generate induced pluripotent stem cells (iPSCs), which can be turned into motor neurons or glia in cell culture. The first ALS patient-derived motor neurons were generated in 2008 by the laboratories of Drs. Kevin Eggan at Harvard and Chris Henderson at Columbia. However, we do not yet know whether these cells are capable of showing ALS disease properties in cell culture. Although this technology is quite promising, it is still very new. Efforts are underway in many labs around the world to develop new ways to create iPS cells and to generate motor neurons and glia that display ALS disease characteristics.

In collaboration with Dr. Chris Shaw's lab in London and Dr. Ian Wilmut's lab in Edinburg, we are characterizing motor neurons and glia derived from iPS cells bearing two recently discovered mutations that, like SOD1, can cause ALS. These are mutations in genes called FUS/TLS and, TDP-43. Drs. Shaw and Wilmut obtained skin cells from ALS patients with mutations in their FUS/TLS or TDP-43 genes, and with the help of Dr. George Daly's lab at Harvard, turned them into iPS cells. We, in turn, have successfully turned these iPS cells into motor neurons and currently are characterizing them.

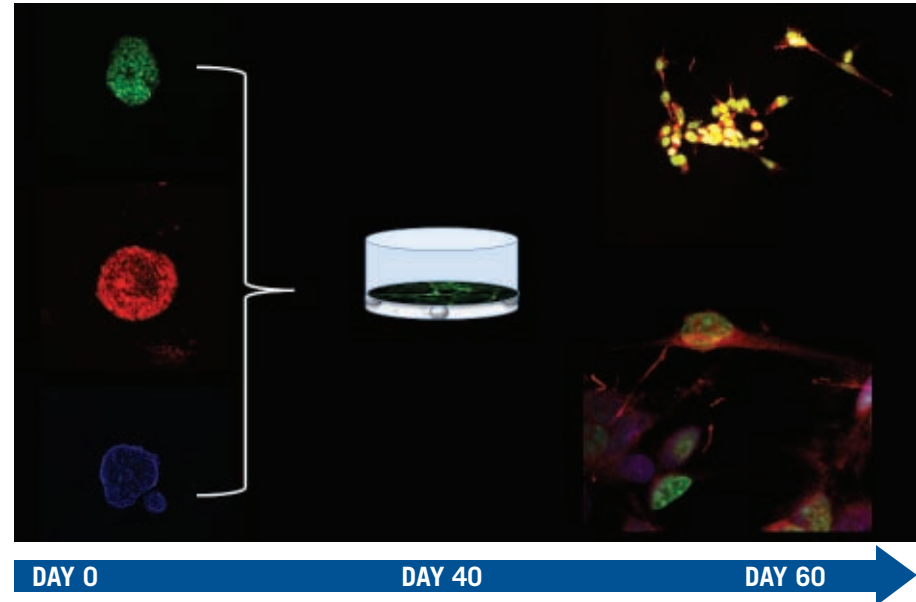
We are using all of the tools at our disposal to investigate the iPS cell-derived neurons. We have optimized the methods used to produce these iPSC-derived motor neurons and use several methods to confirm that

they that are bona fide motor neurons. For instance, we have optimized the time frame of the conversion from iPSCs to motor neurons at four weeks, after which the cells are kept in culture for two to six weeks (Figure 1). Over this time we see maturation and death of motor neurons and we have focused our research on gathering evidence and looking for hallmarks of ALS in this human model.

In addition to these studies, we have used the latest genome technology to monitor changes in the expression of thousands of genes throughout the genome for normal, TDP-43 and FUS/TLS motor neurons generated from ALS-iPSCs. In this way, we are building a more complete picture of diseased cells and the cellular mechanisms that cause motor neuron death in ALS. Motor neurons are like complex machines with many moving parts, and we are searching through the machine to find the malfunctioning parts.

Our studies have focused mainly on the molecular mechanisms that cause ALS because we believe that an understanding of the processes that occur in and around motor neurons in ALS patients is the key to understanding how the disease is caused and how it progresses. Much of our current research is directed towards understanding the role of glia in ALS, and in determining whether motor neurons produced from patient-derived iPS cells can display ALS disease properties. The long-term plan is to create new possibilities for the treatment and cure of this devastating disease.

FIGURE 1



**TIMELINE:** iPSC cell lines from ALS patients are cultured in vitro; these are pluripotent cells that express Nanog (green), Oct 3/4 (red) and SSEA4 (blue). We induce differentiation by treatment with Sonic hedgehog agonist and retinoic acid. After four weeks we plate the already differentiated cells and we keep them in culture between four to six weeks. Motor neurons are shown in the right; image shows tuji1 (red), HB9 (green) and Dapi (blue).

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case of mutant SOD1, understanding the mechanism underlying the translocation of the mutant protein to the mitochondria may begin to answer this question since it would provide a mean to test whether preventing mutant SOD1 association with the mitochondria alters ALS pathogenesis. Uncovering this mechanism also opens new possibilities for therapeutic intervention in addition to those aimed at

improving mitochondrial function. Finally, thanks to recent advances, such as the discovery of new ALS linked mutations in TDP-43 and FUS/TLS and the use of cellular models to mimic sporadic ALS pathogenesis, the role of the mitochondria can now be studied in these models, thus furthering our understanding of the pathogenic mechanisms in ALS.

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*Animal studies combining CNTF and BDNF demonstrate decreased motor neuron loss*  
*GDNF rescues degenerating motor neurons during development in an in vitro experiment*

## timeline cont.

1991

Researchers link familial ALS to chromosome 21

The ALS Association begins workshops

1992

Glutamate transporter shown to be defective in ALS  
 Growth factor BDNF found to increase survival of motor neurons

1993

SOD1 gene mutation (chromosome 21) discovered in familial ALS  
 Trials using glutamate blocker riluzole begin

1994

Transgenic animals carrying mutated human SOD1 gene exhibit ALS-like symptoms and pathology

1995

FDA approves riluzole

1996

Toxic properties of the SOD1 enzyme discovered and linked to familial ALS

1997

# Models of TDP-43

Continued from page 1

made to overexpress TDP-43. To investigate TDP-43 pathology in humans, postmortem brain and spinal tissue is examined, essentially providing a snapshot of TDP-43 abnormalities at the end-stages of disease.

At this point, TDP-43 fragments are primarily insoluble, suggesting that they are components of TDP-43 inclusions. However, little is known about how or when these fragments are generated and what role, if any, they play in the disease process.

Since animals can be sacrificed at different time-points during disease, they provide an ideal tool with which to examine these questions. For example, in mice overexpressing mutant TDP-43, fragments were observed in the brain and spinal cord prior to the onset of gait abnormalities and brain pathology [1]. Similarly, a correlation between disease progression and the accumulation of TDP-43 fragments was observed in other TDP-43 transgenic models [2, 4].

Overall, these results suggest that TDP-43 fragments play a role in TDP-43-associated neurotoxicity. What is quite interesting is that the TDP-43 fragments observed in the transgenic animals were mostly present in a soluble, and presumably non-aggregated, form. This raises the possibility that TDP-43 fragments are toxic in and of themselves, and not necessarily because they assemble into inclusions.

While the initial characterization of TDP-43 transgenic animals might have focused on whether TDP-43 forms inclusions and

becomes abnormally phosphorylated, ubiquitinated or truncated, other notable features were discovered. For instance, Xu and colleagues found that their TDP-43 transgenic mice developed clusters of abnormal mitochondria in the cytoplasm of spinal cord motor neurons. The energy supplied by mitochondria is essential for proper neuronal function and survival. The clustering of mitochondria, although unexpected, was verified by a second model further suggesting that this abnormality may play a role in TDP-43 disease pathology [7]. Indeed, it is noteworthy that mitochondria-mediated toxicity is believed to contribute to the pathogenesis of ALS and various other neurological diseases.

Given that TDP-43 inclusions were not always observed in the above-mentioned models, and given that the redistribution of TDP-43 from the nucleus to the cytoplasm was modest, these models are not necessarily ideal for assessing how loss of TDP-43 function contributes to neurotoxicity. Rather, the use of transgenic mice which express no TDP-43 would be a preferable approach for studying this question. Unfortunately, TDP-43 is essential for early mouse embryogenesis, making the creation of such mice difficult. Mouse models are now emerging in which TDP-43 is deleted after birth. Not only will such models help decipher what functions are carried out by TDP-43 in the central nervous system, they will prove valuable in determining if loss of TDP-43 function contributes to ALS.

Models	Human A315T TDP-43 overexpressed in mice with high levels in brain and spinal cord. [1]	Human wild-type TDP-43 overexpressed in mice in virtually all neurons. [2]	Mouse wild-type TDP-43 overexpressed in mice primarily in hippocampus, cortex and striatum. [3]	Human A315T TDP-43 overexpressed in mice primarily in brain and spinal cord. [4]	Human M337V TDP-43 ubiquitously overexpressed in rat. [5]	Human wild-type TDP-43 overexpressed in mice primarily in brain and spinal cord. [6]	Human wild-type TDP-43 overexpressed in mice in virtually all neurons of the CNS. [7]
<b>Promoter driving TDP-43 expression</b>	Mouse prion protein promoter	Mouse Thy-1 promoter	Ca <sup>2+</sup> calmodulin kinase II promoter	Mouse prion protein promoter	Conditional expression of TDP-43 using the Tet-Off system (TRE-TDP-43 rats were crossed with CAG-Tta rats)	Mouse prion protein promoter	Mouse Thy-1.2 promoter
Increased ubiquitin levels or inclusions	+	+	+	+	+	+	+
Cytoplasmic TDP-43 inclusions	-	+	+	+	+	+	-
Nuclear TDP-43 inclusions	-	+	-	+	-	+	+
Fragmented TDP-43	+	+	+	+	+	+	-
Phospho-TDP-43	not investigated	+	-	+	+	+	-
Gliosis	+	+	+	+	+	+	+
Axonal degeneration and/or neuron loss	+	+	+	not investigated	+	+	-
Motor impairment (e.g. gait abnormalities, paralysis)	+	+	+	+	+	+	+
Cognitive dysfunction	-	-	+	-	-	-	-
Shortened lifespan	+	+	+	+	+	+	+

TABLE 1

Even though our understanding of TDP-43 remains incomplete, great advances have been made in a relatively short amount of time. The novel animals models described herein provide much needed tools with which to answer the many questions regarding TDP-43 function and dysfunction. Since TDP-43 pathology is observed in various neurodegenerative diseases, it has evoked widespread interest. Hopefully, this should hasten the advances made regarding the role played by TDP-43 in disease progression and provide insight on new therapeutic approaches for ALS.

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*Study shows surrounding support cells play key role in ALS*

*Study shows that human embryonic stem cells can be stimulated to produce motor neurons*

*Gulf War study shows that vets deployed to Persian Gulf in 1991 developed ALS at twice the rate of those not deployed there*

*IGF-1 gene therapy study proves beneficial in mice with ALS*

*VEGF gene abnormalities shown to be potential factor in ALS*

*The ALS Association collaborates with U.S. Department of Veterans Affairs to enroll all vets with ALS in registry*

**2003**  
*Early tests of ceftriaxone appear to increase survival in mice with ALS  
 Combination of creatine and minocycline prove more effective together in mouse model than either drug alone*

*A transgenic rat is designed; efforts start on fly model*

*Attention turns to support cells of nerve tissue to find role in ALS*

*Inflammation and programmed cell death gather research interest*

*ALS2 gene (alsin protein) linked to juvenile ALS*

**2001**  
*The ALS Association/NINDS collaborative effort begins screening drugs*

*The ALS Association holds scientific workshop on "Environmental Factors and Genetic Susceptibility"*

*Aggressive search for new ALS genes funded by The ALS Association*

*Scientists complete map of mouse genome*

*Agency of Toxic Substances and Disease Registries awards five grants focused on ALS*

**2002**  
*Department of Defense approves funding for ALS-specific research*

*The ALS Association co-sponsors workshop on high-throughput drug screening with NINDS*

**2000**  
*NINDS issues first ever RFA (request for applications) specifically for ALS research*

## timeline cont.

**1998**  
*RNAi discovered by Craig Mello and Andrew Fire*

**1999**

*RNAi discovered by Craig Mello and Andrew Fire*

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# The Milton Safenowitz Post-Doctoral Fellowship for ALS Research

Three young investigators funded by The Milton Safenowitz Post-Doctoral Fellowship for ALS Research are engaged in innovative projects to accelerate progress in the field. The ALS Association is especially committed to bringing new concepts and methods into ALS research, and young scientists play an important role in this process. Funding is generously provided by the Safenowitz family through the Greater New York Chapter of The ALS Association, in memory of Milton, who died in 1998 of the disease.

## Whole exome capture and parallel DNA resequencing of Familial ALS cases

Hussein Daoud, Ph.D., Center of Excellence in Neuromics, Montreal, Canada



Hussein Daoud, Ph.D.

*"I am honored and grateful to receive the Milton Safenowitz Post-Doctoral Fellowship from The ALS Association. ALS is a neurodegenerative disease that is still not completely understood and has no treatment or cure. The goal of my post-doctoral training is to identify new causative ALS genes. For those who are living with this devastating disease, this would be of tremendous help as it would open new avenues for research into the pathogenesis of ALS and would certainly have a substantial impact on the best approach to choose for the development of new therapies. This fellowship will help me finance my post-doctoral training on the genetics of ALS and will create opportunities for me to keep up my fight against this disease."*

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that leads to a progressive paralysis due to the death of large motor neurons in the brain and spinal cord. While the discovery of several genes, notably SOD1, TARDBP and FUS has led to significant new insights into the causes of ALS, the basic pathogenic mechanism and the genetic etiology of most ALS cases remain unknown. Most important, the genetic cause of more than 50% of familial ALS cases (FALS) remains to be identified.

Therefore, it remains important to identify additional ALS-causing genes and converging evidence suggests that a large number of rare, highly pathogenic mutations underlie a substantial fraction of the familial form of the disease.

Such rare mutations cannot be identified using whole genome association methods as their identification requires a direct resequencing of patient DNA. The investigators propose to use modern and powerful DNA sequencing methods available to their group and expand the genetic screen analysis to the entire coding genome by resequencing all the coding exons, or "exome," in a cohort of 60 unrelated SOD1, TARDBP and FUS negative FALS patients (and unaffected relatives), which will be selected from their largest and clinically well-defined affected families.

## Understanding the landscape of neuroinflammation and identification of key players using MCP1-CCR2-SOD1G93A triple transgenic mice in vivo

Javier Jara, Ph.D., Northwestern University, Chicago, Ill.



Jara Javier, Ph.D.

*"I am honored to receive The Milton Safenowitz Post-Doctoral Fellowship. I am currently focusing on understanding the cell-type specific aspects of neuroinflammation and its effects to both upper and lower motor neurons during disease initiation and progression in ALS. Using genetic labeling approaches, coupled with fluorescence activated cell sorting, the work supported by this fellowship will be instrumental in the characterization of the key players of neuroinflammation in ALS."*

In ALS motor neurons in the cortex, brainstem and spinal cord show vulnerability and progressively degenerate. Cells that initiate an immune response both locally and broadly in the system are suggested to play a role in ALS disease. These cells secrete molecules called cytokines, which have a wide variety of functions from immune response to

*Continued on page 8*

## timeline cont.

Study implicates smoking as likely risk factor in sporadic ALS  
Study releases evidence that mitochondrial malfunction may play an important role in ALS

Ceftriaxone increases levels of the glutamate transporter GLT1 in a mouse model of ALS

First international workshop on frontotemporal dementia discusses link to ALS

Stem cells engineered to make GDNF survive when transplanted into rats modeling ALS

Early data suggests that mutant SOD1 may be secreted by and may activate microglia

Launch of TREAT ALS initiative (Translational Research Advancing Therapies for ALS) to accelerate clinical trials in ALS

ALS patient samples collected to NINDS ALS Repository

Repository samples allow genome analysis for sporadic ALS

First TREAT ALS clinical trials funded

First TREAT ALS clinical trials begun

Stem cell study shows SOD1 mutant support cells can kill any motor neuron

ALS U.S. registry efforts gaining ground in Congress

Fish model of ALS: Progress reported

SOD1 in altered form common to both sporadic and inherited ALS

Engineered stem cells making GDNF help motor neurons survive in SOD1 mutant rats

Stem cells generated from ALS patients

Discovery of DPP6 in two genome-wide association studies in ALS

Mutations in TDP-43 linked to familial and sporadic ALS

Identification of new gene linked to familial ALS, Fused in Sarcoma (FUS) on chromosome 16

First patients enrolled for antisense and stem cell trials in U.S.

2004

2005

2006

2007

2008

2009

2010

Study funded by The ALS Association to find biomarkers in cerebrospinal fluid and blood

VEGF increases survival in a rat model of ALS while improving motor performance

TDP-43 discovered as a common link in FTD, ALS  
Chromosome 9 region intense focus for FTD, ALS

First genome screening data published based on NINDS ALS Repository

Induced Pluripotent Stem Cell Technology opens up new avenues for ALS

FDA approval of SOD1 antisense and stem cell trials in U.S.

## Biomedical Research

Building an ALS TRANSLATIONAL RESEARCH PIPELINE

### Request for Abstracts (RFA)

The ALS Association Research INVESTIGATOR-INITIATED RESEARCH GRANT PROGRAM supports INNOVATIVE research of high scientific merit and relevance to amyotrophic lateral sclerosis (ALS), offering investigators awards in the following categories:

### Multi-Year Grants

The ALS Association will support research that is projected for periods of up to three (3) years. Funding for multi-year grants is committed for one (1) year only, with noncompetitive renewals conditioned upon results. These applications require strong preliminary data. Awards will be in the amount of up to \$80,000 per year.

### Starter Grants

One-year awards for NEW INVESTIGATORS ENTERING THE FIELD OF ALS. Alternatively, they can be PILOT STUDIES BY ALS INVESTIGATORS. These applications do not require strong preliminary data but must emphasize innovation, scientific merit, feasibility and relevance to ALS. The maximum amount awarded is \$40,000.

### The Milton Safenowitz Post-Doctoral Fellowship for ALS Awards

The maximum amount awarded is \$40,000 per year for two years. Eligibility is limited to those who have been a fellow for one year or less.

Request an abstract form for any of these categories from [researchgrants@alsa-national.org](mailto:researchgrants@alsa-national.org). You will be notified within two weeks of the abstract submission due date whether you are eligible to submit a full application. See schedule below.

<b>Grant Schedule:</b>	2011
<b>Call for Abstracts:</b>	1st December 2010
<b>Abstracts Due:</b>	5th January 2011
<b>Full Application Due:</b>	4rd March 2011
<b>Award Announcements:</b>	June 2011
<b>Funding Commences:</b>	1st August 2011

E-mail: [researchgrants@alsa-national.org](mailto:researchgrants@alsa-national.org)

### RESOURCES

- SOD1 mutations database [www.alsod.iop.kcl.ac.uk/als](http://www.alsod.iop.kcl.ac.uk/als)
- Coriell NINDS DNA repository <http://ccr.coriell.org/ninds/>
- ALS Epidemiology <http://aces.stanford.edu/ForRes.html>
- SOD1 mutant rats, Taconic, <http://www.taconic.com/wmspage.cfm?parm1=258>
- SOD1 mutant mice, The Jackson Laboratory <http://jaxmice.jax.org/models/als.html>
- Control and SOD1 fibroblasts [http://ccr.coriell.org/Sections/Search/Advanced\\_Search.aspx?PgId=175&ct=C&result=y&coll=ND](http://ccr.coriell.org/Sections/Search/Advanced_Search.aspx?PgId=175&ct=C&result=y&coll=ND)
- ALSUntangled <http://www.wfnals.org/alsu.html>

## Safenowitz Post-Doctoral Fellowship

*Continued from page 7*

neuronal protection. We still do not know what exact role these cells have on disease initiation and progression and how cytokines are involved in the process. If we have a clear understanding of the system and the role of each player, we can then build effective treatment approaches in ALS.

The investigators have generated a triple transgenic ALS mouse model, in which the cytokine (MCP-1) and its receptor (CCR2) are genetically labeled with red and green fluorescence, respectively. Using this mouse model they will overcome two major limitations in the field: 1) They will visualize their cellular interactions with the vulnerable motor neurons both in the cortex and spinal cord over time, and 2) they will isolate and purify them to further investigate and identify the subset of molecules that are involved in local and broad immune reaction in ALS. This is novel and very important for the future development of treatments.

### Determining non-cell autonomous contributions of TDP-43 and FUS mutations in ALS using embryonic stem cells

Dara Ditsworth, Ph.D., Ludwig Institute for Cancer Research, UCSD, San Diego, Calif.

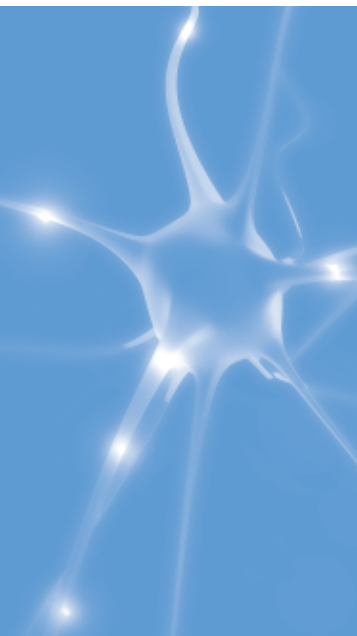


Dara Ditsworth, Ph.D.

*"My research focuses on defining the cell autonomous vs non-cell autonomous properties of TDP-43 and FUS mutations in cell types relevant for ALS. By differentiating mouse embryonic stem cells into either motor neurons or glia, I am investigating the effects of gain or loss of function of these genes in purified cell populations. I am so excited to be awarded a Milton Safenowitz Post-Doctoral Fellowship from The ALS Association, which will allow me to broaden my research expertise from basic cell biology and biochemistry to utilize stem cells as a model for neurodegeneration."*

The recent identification of mutations in TDP-43 and FUS in both sporadic and familial cases of ALS suggests that alterations in RNA processing may play a pivotal role in ALS pathogenesis. In addition to these mutations, TDP-43 and FUS have been identified as components in the pathology of several neurodegenerative diseases. Whether disease-associated mutations lead to the loss of an essential normal function or the gain of a toxic new function remains unclear. Furthermore, whether mutations in TDP-43 or FUS affect other cell types that contribute to motor neuron disease progression is unknown.

The investigators aim to explore consequences of the loss or gain of function mutations in TDP-43 and FUS by using embryonic stem cells from existing transgenic mice and inducing differentiation into motor neurons or glia. Combined with cutting-edge technology to analyze global alterations in gene expression, these aims will address basic questions in isolated cell populations or in mixed cultures of motor neurons or glia. It is their hope that this work will lead to greater understanding of disease mechanism, and may provide rationale for future studies of whether manipulation of supporting glial cells would provide therapeutic benefit in patients with mutations in TDP-43 or FUS.



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