

RESEARCH ALS TODAY

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Major Gene Discoveries Bring Us Closer to Developing More Promising Treatments for ALS

Lucie Bruijn, Ph.D.



Teepu Siddique, M.D.

In the last two months, the field of ALS research has seen some major discoveries that will significantly impact how we understand the disease. Two new genes linked to familial ALS have been identified. Although a great deal more work needs to be done to understand how mutations in these genes lead to ALS and dementia, these findings will provide new directions for drug discovery and the development of treatments that will alter the course of the disease.

Investigators led by Dr. Teepu Siddique at Northwestern University Feinberg School of Medicine identified a new gene UBQLN2 which encodes the ubiquilin-like protein, ubiquilin-2, linked to familial ALS with and without dementia. The protein is involved in the degradation of ubiquitinated proteins, providing further support that protein mishandling is an underlying mechanism involved in ALS. The mutations, first identified in a single chromosome X-linked family and later identified in four additional unrelated families, are autosomal dominant with an onset in disease varying between 16 and 71 years of age.

Closely following this major discovery was the identification of a GGGGCC hexanucleotide

repeat within the non-coding region of a gene on chromosome 9p21 called C9ORF72 by two independent teams, one led by Bryan J. Traynor, M.D., Laboratory of Neurogenetics, National Institute on Aging, and the other by a group at Mayo Clinic in Jacksonville, Florida led by Rosa Rademakers, Ph.D., both funded by The ALS Association.

In healthy individuals this hexanucleotide is repeated only two to 23 times, but in ALS or frontotemporal dementia (FTD) patients it is repeated 700-1,600 times. This repeat accounts for nearly 50% of familial ALS cases in Finland and more than a third of familial cases in other European populations. These changes were found in almost 12 percent of familial FTD and more than 22 percent of familial ALS samples studied at Mayo Clinic. The defect is also the strongest genetic risk factor found to date for the more common, non-inherited, sporadic forms of these diseases. It was found in three percent of sporadic FTD and four percent of sporadic ALS samples in Mayo Clinic's large clinical patient series. The repeat expansion is more than twice as common as the SOD1 gene in familial ALS

and four times as common as TDP-43, FUS, VCP combined. The identification of this repeat may have immediate utility by allowing early identification of ALS patients at risk of cognitive impairment and FTD cases at risk of progressive paralysis.

The investigators believe that when the defective gene is transcribed into a messenger RNA molecule, the expanded repeat section causes the RNA to bind tightly to certain proteins, forming accumulations within the brain cells. By binding these proteins, the abnormal RNA may prevent these proteins from carrying out their normal functions in the cell. Disruption of RNA metabolism has already been identified as an important mechanism in those cases with TDP-43 and FUS mutations, and this discovery provides further evidence for disrupted RNA metabolism as a key underlying cause of disease.



Bryan J. Traynor, M.D.



Rosa Rademakers, Ph.D.

For additional exciting new findings see *Journal News* on page 8.

ABSTRACT DEADLINE
January 6, 2012

See page 6



Landmark Gene Findings Pave the Way for Significant Progress in ALS Research

These last months have seen remarkable discoveries for ALS that will dramatically change our understanding of the disease. Highlighted in this edition are the new gene mutations linked to familial and some sporadic cases of ALS providing compelling evidence that RNA metabolism plays a pivotal role in disease mechanisms. This is the first time that expanded repeats are associated with ALS; these findings add to a growing number of repeat disorders in neurodegeneration. It is also the first genetic link between ALS and frontotemporal lobar degeneration (FTLD).



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

These recent studies will encourage new investigators to enter the field of ALS research and will provide opportunities for collaborations amongst experts in related disorders to understand how the hexanucleotide repeats cause disease, the links between FTLD and ALS, and the potential for therapeutic development.

Research continues to focus on unraveling the roles of TDP-43 and FUS in ALS and the challenges of building the appropriate model systems to study disease mechanism and develop treatments. With additional mutations, there will be a wealth of new model systems being developed, each bringing us closer to a better picture of what goes wrong in ALS. Over 50% of all the familial genes have now been identified. The pace of new discoveries is encouraging, and it is our hope that the remainder of the genes will be identified in the near future especially with the advances in technology such as whole exome sequencing.

The ALS Association is committed to supporting researchers in these important studies as well as bringing scientists and clinicians together to form new collaborations and bring novel ideas to the table. We are encouraged by the increasing number of investigators entering the field of ALS and continue to encourage young scientists to pursue ALS research. This edition highlights numerous new studies and research fellows pursuing a variety of important research topics, and we encourage researchers to apply for funding through our upcoming grant round.

—*Lucie Bruijn, Ph.D.*

The TREAT ALS™ Research Portfolio Supports a Diverse Range of New Research Studies

Barbara Bronson-Gray

TREAT ALS (**T**ranslational **R**esearch **A**dvancing **T**herapies for **ALS**) is an international effort catalyzing research critical to understanding and treating ALS—and finding a cure.

Partnering with academia and industry to test the potential of new therapies and support the infrastructure for clinical trials, the TREAT ALS-supported research is designed to help find the cause or causes of ALS, determine the mechanisms involved in the progression of the disease, and discover effective treatments.

The grant recipients this year represent a diverse combination of researchers based in the United States, Belgium, Italy, Spain and the United Kingdom. The new grants tackle a wide range of research challenges, building on previous work and expanding new frontiers of discovery.

For example, several studies aim to explore TDP-43, a gene that is a relatively common cause of familial ALS and has appeared in some people with sporadic ALS. Ultimately, the

Continued on page 3

“The new grants tackle a wide range of research challenges, building on previous work and expanding new frontiers of discovery.”

The TREAT ALS™ Research Portfolio

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TDP-43-related research may also help researchers find biomarkers for ALS and offer a better understanding of mechanisms that lead to neurodegeneration in people with ALS.

Others are also working to identify biomarkers, biological signals that can be used to definitively diagnose ALS and correlate with the progression of the disease. Some data indicates that microRNAs in mutant SOD1 mice can be detected in circulating plasma. (MicroRNAs are small regulatory molecules that bind specific cellular messenger RNAs [mRNA] and inhibit the translation of that mRNA to protein). The research could lead to the discovery of valuable biomarkers for ALS detection.

Several other studies promise to advance our understanding of SOD1 mutations toxic to motor neurons and responsible for a subset of ALS cases. The SOD1 gene produces the SOD1 enzyme, which reduces free radicals in cells. But the association between SOD1 mutations and motor neuron death is not yet well understood. Studies may show the critical missing links between the damaged gene and the pathological process that contribute to ALS.

Other studies explore the role of cells other than motor neurons that could be causing neural damage. Astroglial cells, or astrocytes—perhaps in association with the SOD1 gene—are sometimes found to be ineffective in clearing toxic glutamate away from motor neuron synapses. They can contain unusual aggregations of protein that increase as the disease progresses. Motor neurons may be more prone to damage when there are damaged astrocytes in the cellular neighborhood.

Some researchers are looking at TDP-43, SOD1 and FUS/TLS mutations together in both familial and sporadic ALS. FUS is a multifunctional protein similar to TDP-43 and is involved in RNA metabolism.

Researchers will be evaluating the feasibility of using stem cells in the treatment of ALS. One group of investigators will test the ability or progenitor cell grafts that produce and secrete growth factors to enhance the delivery of the growth factors directly to skeletal muscles in rats with the SOD1 gene. Their work will show whether a new line of skeletal muscle progenitor cells—which act as a repair system for the body—can enhance, or even replace, established mechanisms of stem cell delivery in skeletal.

Others are studying the immune system to understand the role inflammation may play in the disease. Researchers have

found that a type of white blood cells, called monocytes, can enter the spinal cord and cause damage to the motor neuron in ALS. They have found a way to reduce the inflammatory properties of the monocytes and reduce their infiltration into the spinal cord, causing animals to live longer and have less spinal cord damage. They're now going to delve deeper into how that treatment leads to prolonged survival in mice and study the blood and spinal fluid from patients with ALS to better understand what inflammatory changes can be detected. Because the changes can be noted at very early stages of ALS in animals, it is hoped that the research could lead to detection of ALS in humans in its earliest stages as well.

Other investigators are also interested in detection mechanisms associated with the spinal cord, looking for early signs of malfunction in its synaptic circuits. A group of researchers have developed a genetic biosensor they will be testing in mouse models of ALS. The technology will allow scientists to monitor and see in real time the activity of synapses in the diseased nervous system, facilitating the analysis of early pre-symptomatic events in ALS. Their work could potentially lead to the development of biological and pharmacological treatments for prevention or intervention in the disease.

“Other investigators are also interested in detection mechanisms associated with the spinal cord, looking for early signs of malfunction in its synaptic circuits.”

Some researchers are investigating how the efficacy and impact of the only licensed therapy for the treatment of ALS, riluzole (Rilutek®) could be improved. By modifying the chemical structure of riluzole and generating hundreds of derivatives of the drug, they will analyze the “spin-offs” to assess their ability to protect motor neurons, looking for the most effective derivations. The process may also help researchers better understand the precise mechanism through which riluzole—in its currently-used formulation—targets cells. The goal of this effort will be to generate a drug-like molecule that could lead to a more effective therapeutic treatment of ALS.

Taken together, these research projects, funded by The ALS Association, promise to better understand the disease mechanisms in ALS and discover the most promising strategies to slow, stop and treat ALS disease progression.

For more details see website link:
<http://www.alsa.org/news/archive/2011-new-research-grants-1.html>

Blame it on RNA Processing

by Clotilde Lagier-Tourenne, Ph.D.
Milton Safenowitz Post-Doctoral Fellow



Lagier-Tourenne, Ph.D.

Amyotrophic Lateral Sclerosis

(ALS) research is at a very exciting period with the identification of new disease-causing genes pointing out the importance of RNA processing regulation in ALS pathogenesis.

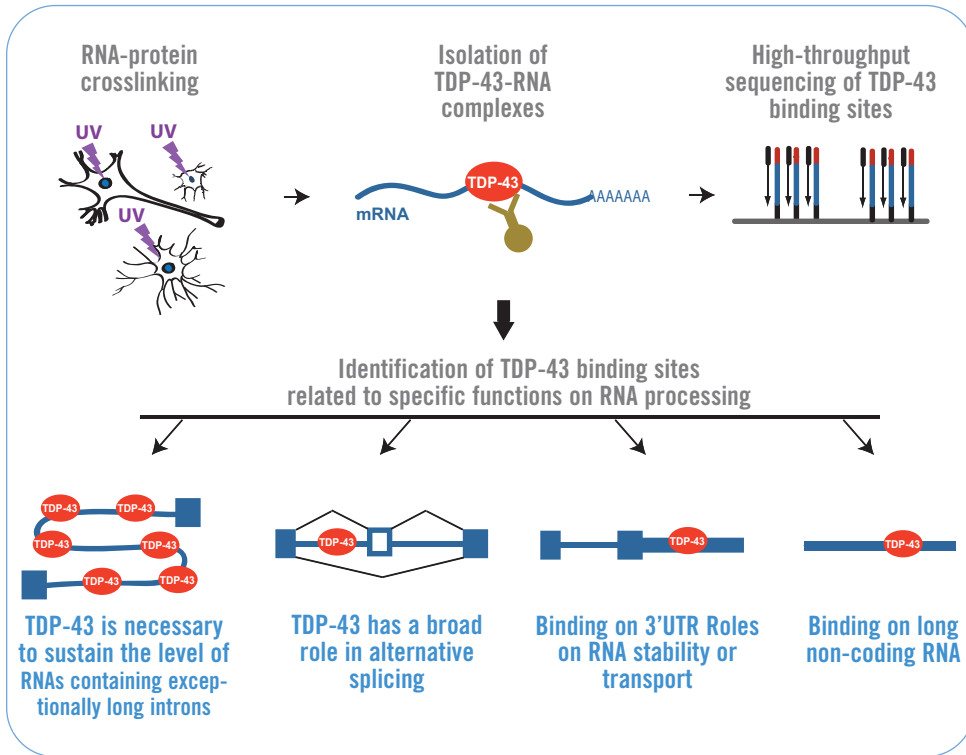
Ribonucleic acids (RNAs) have multiple functions in the cell including the essential role of messenger RNA (mRNA) to carry the genetic information from the nucleus to the cytoplasm to serve as a template for protein synthesis (also called translation). RNA processing (synthesis, maturation, transport, translation or degradation) is highly regulated and involves multiple DNA/RNA binding proteins to determine proper gene expression.

Mutations and/or cellular mislocalization of two DNA/RNA binding proteins, TDP-43 and FUS/TLS, have been identified as central components in the pathogenesis of ALS. Several lines of evidence suggest that both proteins are involved in multiple steps of RNA metabolism including transcription (synthesis of RNA), splicing (maturation step to remove the introns from RNA) and RNA transport, yet which of these roles are the most important within the nervous system is not known. Mechanisms by which TDP-43 and FUS/TLS trigger neurodegeneration are only at the earliest phases of investigation, and it is unresolved as to whether neurodegeneration is due to a loss of TDP-43 or FUS/TLS function, a gain of toxic property, or a combination of the two arising

from their sequestration into nuclear or cytoplasmic aggregates. TDP-43 is mainly detectable in the nuclei in unaffected neurons, but it is partially cleared from those nuclei in neurons containing cytoplasmic aggregations, supporting that pathogenesis is driven, at least in part, by a loss of TDP-43 nuclear function. A similar pattern has also been observed in presence of FUS/TLS aggregates. Importantly, abnormal RNA processing has been reported in sporadic ALS patients, albeit a role of TDP-43 or FUS/TLS in these modifications has not been explored. It is now crucial to determine the normal functions of TDP-43 and FUS/TLS and to identify the set of alterations in RNA processing that define a TDP-43 and FUS/TLS-dependent disease signature.

A set of experiments using revolutionary sequencing technologies have recently provided the first insights on the normal functions of TDP-43 within the central nervous system. Until recently, only candidate approaches could be used to identify specific RNA-binding proteins' targets or aberrant splice isoforms related to diseases. Advances in DNA sequencing technology have provided powerful tools for exploring gene regulation in remarkable detail. Indeed, by providing millions of sequences at the same time, this technology is revolutionizing our ability to identify modification of the transcriptome in an unbiased manner. It is now possible to isolate an RNA-binding protein in complex with its RNA targets

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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	1989: The ALS Association funds search for a common genetic link to ALS	1990: Growth factor CNTF is found to increase survival of motor neurons
														1985: The ALS Association funds study of inherited motor neuron disease	1990: Congress declares the 1990s the "Decade of the Brain"	
														1986: Genes for muscular dystrophy identified		

RNA Processing

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and to precisely identify the binding sites within target mRNAs that can be related to a specific function. Contrary to candidate approaches, this method has the potential to identify previously unknown functions of the RNA-binding proteins.

While only a handful of RNA targets were previously known for TDP-43, methods linked to high-throughput sequencing recently identified several thousands of TDP-43 RNA targets defining an RNA-protein interaction map and demonstrating the broad role of the protein in RNA processing (Polymenidou *et al.*, 2011; Sephton *et al.*, 2011; Tollervey *et al.*, 2011). Consistent with *in vitro* studies, *in vivo* TDP-43 binding sites were enriched in sequences containing stretches of uracil guanine (UG) repeats. The vast majority of the binding sites were found within introns but usually far away from intron-exon junctions, which are the boundaries used to remove introns during splicing. This finding was surprising for a protein that was best characterized for binding close to an intron-exon junction and regulating a splicing event on the gene mutated in cystic fibrosis (CFTR).

Furthermore, this sequencing approach—and another unbiased approach in which thousands of splice junctions could be examined simultaneously—established that TDP-43 has a broad role in alternative splicing. Reduction of TDP-43 within the mouse central nervous system also established that TDP-43 is required to sustain the level of RNAs containing exceptionally long introns, a

feature that was found to be most prominent within brain-enriched transcripts. Strikingly, several of these genes have a crucial role in synaptic activity and function and have previously been implicated in neurological diseases such as Parkin (Park2), Neurexin 1 and 3 (Nrxn1, Nrxn3) or Neuroigin 1 (Nlgn1).

Although most binding sites were found in introns, it is noteworthy that more than a thousand RNAs were bound in 3'-untranslated regions (3'-UTR) at the end of the corresponding RNAs, consistent with multiple roles of TDP-43 on RNA processing in the nucleus and in the cytoplasm. Indeed, binding onto 3'-UTRs has been linked with a role in RNA stability or transport for local translation. RNA transport is particularly crucial in neurons for the local production of proteins in dendritic synapses, where neurons communicate with each other. TDP-43 binding sites on 3'-UTRs and its presence in dendritic spines upon stimulation support that TDP-43 may have a role in RNA transport and local translation in synapses. Interestingly, binding of TDP-43 was identified on the 3'-UTR of several genes involved in ALS pathogenesis such as FUS/TLS, the light chain of neurofilament (NFL) or the glutamate transporter EAAT2. It was also found that TDP-43 binds the 3'-UTR of its own mRNA to tightly auto-regulate its level (Ayala *et al.*, 2011; Polymenidou *et al.*, 2011). This has led to an attractive model that disruption of TDP-43 auto-regulation may lead to a feed-forward mechanism driving disease progression in ALS patients. Another feature uncovered by unbiased

approaches linked to high-throughput sequencing is the binding of TDP-43 onto several long, non-coding RNAs. These RNAs do not serve as template for the production of proteins and can have multiple roles in the cells, most notably the regulation of gene expression. Further studies will be crucial to determine if specific long non-coding RNAs play a role in ALS pathogenesis. Interestingly, TDP-43 has been proposed to affect the levels of another type of non-coding RNA, called microRNAs, and efforts are underway to determine if specific TDP-43-regulated microRNAs play a role in ALS pathogenesis.

It is now crucial to identify which of TDP-43 regulated events are altered in ALS patients. Similar approaches using high-throughput sequencing are underway to identify FUS/TLS RNA targets and RNA processing changes upon FUS/TLS depletion. Deciphering the function of both TDP-43 and FUS/TLS will determine if these two RNA-binding proteins may act through common pathways to trigger and/or exacerbate the progression of neuronal death.

Involvement of TDP-43 and FUS/TLS in ALS reinforces the emerging view that RNA processing is a central feature of motor neuron degeneration, as already demonstrated in spinal muscular atrophy. The role of RNA metabolism in neurodegeneration was further underscored by the recent recognition of intermediate length polyglutamine expansions in ataxin-2, another RNA-binding protein, as a

risk factor for ALS (Elden *et al.*, 2010). Even more recently, a breakthrough in the genetics of ALS occurred with the discovery of an abnormal repeat expansion on chromosome 9 as the most common genetic cause of ALS.

Further studies will be necessary to determine how this expansion in a non-coding region (the first intron) of the gene C9orf72 triggers disease. However, the nature of the mutation echoes a mechanism via RNA toxicity with a loss of function of RNA-binding proteins being trapped by the expansion, as already established in other neurological diseases such as myotonic dystrophy. If a similar mechanism is demonstrated in ALS, it will further emphasize the crucial role of RNA misregulation in ALS pathogenesis. It is our hope that recognizing disease relevant RNA processing alterations will open opportunities for interventional strategies.

Citations

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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

Call for Research Abstracts Due January 2012

BIOMEDICAL RESEARCH

Building an ALS Translational Research Pipeline

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 Research Awards: The maximum amount awarded is \$50,000 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. 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.

Grant Schedule

Call for Abstracts	December 2, 2011
Abstracts Due	January 6, 2012
Full Application Due	March 2, 2012
Award Announcements	July 2012
Funding Commences	August 1, 2012

E-mail: researchgrants@alsa-national.org

Milton Safenowitz Post-Doctoral Fellowships

Five 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.

Determining damage within specific cell types caused by SOD1, TDP-43 and FUS/TLS mutations

Shuying Sun, Ph.D., Ludwig Institute for Cancer Research, UCSD, San Diego, CA



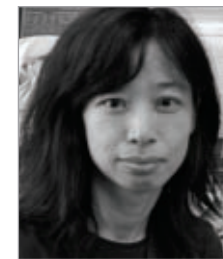
Shuying Sun, Ph.D.

"I am honored to receive the Milton Safenowitz Post-doctoral Fellowship. My research goal is to elucidate how mis-regulation of RNA processing leads to neurodegeneration in ALS, and how specific cell types contribute to the disease pathogenesis. I believe the project supported by this fellowship will not only expand my research expertise in neurodegenerative diseases, but will also provide mechanistic insights into the pathogenesis of ALS."

The investigators will discover the damage done by three types of neurons (motor neurons, astrocytes and oligodendrocytes) by identifying the global translational mRNA changes caused by SOD1, TDP-43, and FUS/TLS ALS-linked mutants within individual cell types. They will also determine the potential concurrent changes caused by the three different mutant genes, potentially providing a test for whether most instances of ALS have common steps that could be targeted to slow disease.

The role of human iPSC-derived NG2 glia on human motor neuron survival: strong implication of NG2 glia in pathogenesis

Ying Li, Ph.D., Johns Hopkins School of Medicine, Baltimore, MD



Ying Li, Ph.D.

"I am honored to receive the Milton Safenowitz Post-doctoral Fellowship for ALS Research. To expand our knowledge of human NG2 glia in ALS, I will differentiate ALS patient specific-induced pluripotent stem cells (iPSCs) to NG2 glia and characterize them. Furthermore, the effects of patient specific iPSC-derived NG2 glia on human motor neuron survival will be determined."

Animal studies indicate that expression of mutant SOD1 in motor neurons determines disease onset, while expression of SOD1 mutations in astrocytes and microglia cells affects disease progression. Investigators recently discovered in ALS mouse models that expression of mutant SOD1 in NG2 glia produces neural disease. Investigators will characterize NG2 glia derived from ALS patient-specific induced pluripotent stem cells (ALS-iPSC) carrying the A4V mutation and investigate the effects of these AV4-mutated NG2 glia on the survival of motor neurons also derived from ALS-iPSC. This study will show whether mutant SOD1 has effects on human NG2 glia biology—and importantly—whether human NG2 glia cells play a role in ALS.

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timeline cont.

1998	1999	2000	2001	2002	2003
RNAi discovered by Craig Mello and Andrew Fire	The ALS Association co-sponsors workshop on high-throughput drug screening with NINDS	NINDS issues first ever RFA (request for applications) specifically for ALS research	The ALS Association/NINDS collaborative effort begins screening drugs	Department of Defense approves funding for ALS-specific research	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*

*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*

*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*

Milton Safenowitz

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Phenotypic characterization of purified motor neurons from ALS patient specific induced pluripotent stem cells

Ole Wiskow, Ph.D., Harvard University Stem Cell and Regenerative Biology, Cambridge, MA



Ole Wiskow, Ph.D.

*"I feel honored and thankful to receive the Milton Safenowitz Post-doctoral Fellowship from the ALS Association. This support will allow me to pursue my research developing new tools to model ALS disease progression **in vitro** using both familial and sporadic ALS patient-specific induced pluripotent stem cell (iPS) lines. Our hope is that such **in vitro** approaches will enable us to further dissect the molecular causes of ALS and facilitate the discovery of new therapeutic targets in the fight against this terrible illness."*

The researchers will create an ALS disease model using human cells capable of creating motor neurons in culture. To facilitate the identification of stem cell-derived motor neurons in culture, the investigators will introduce a motor neuron-specific genetic reporter into the stem cells. The process will make the affected motor neurons accessible for a range of molecular biological analyses and make it possible to directly compare ALS-affected motor neurons with unaffected ones from healthy individuals. The goal is to identify unknown determinants responsible for ALS motor neuron degeneration. Knowledge of such key disease-related cellular pathways could contribute to the development of new drugs that specifically target these pathways, potentially decreasing or stopping motor neuron degeneration in ALS.

Focal transplantation of human neural progenitor cell-derived astrocytes isolated from ALS patients into the rat spinal cord

Amanda Phillips, Ph.D., Department of Neurology, Johns Hopkins University, Baltimore, MD



Amanda Phillips, Ph.D.

*"I am honored to receive the Milton Safenowitz Post-doctoral Fellowship to investigate the **in vivo** biology of human ALS patient-derived astrocytes. This post-doctoral fellowship has allowed me to expand my research in the Maragakis laboratory to examine the **in vivo** biology of these patient-derived astroglia after transplantation to the rodent spinal cord. Since there are currently no **in vivo** models of sporadic ALS, and the cause for disease is unknown, our transplantation-based model affords the unique opportunity to study sporadic ALS astrocyte biology in an **in vivo** context."*

Astrocytes significantly influence the progression of paralytic disease in a mouse model of familial ALS. When diseased astrocytes are replaced with healthy ones, the mice have increased survival. But the role of astrocytes in human sporadic ALS is still unclear. The investigators have isolated astrocytes post-mortem from patients with both familial and sporadic ALS and from healthy controls to look for potential differences between diseased and non-diseased astrocytes. The investigators will inject these cells into the spinal cord of healthy rats and examine their interactions with motor neurons to determine whether the human astrocytes from familial or sporadic ALS patients damage healthy motor neurons in the rat spinal cord. The findings will provide further evidence whether astrocyte-targeted therapies could be developed for sporadic ALS.

Identification of pathogenic TDP-43 targets *in vivo*

Mercedes Prudencio, Ph.D., Research at Mayo Clinic in Florida, Jacksonville, FL



Mercedes Prudencio, Ph.D.

"I am extremely grateful and honored to receive the Milton Safenowitz Post-doctoral Fellowship for ALS Research. The goal of my project is to identify key RNA targets regulated by TDP-43 that may be aberrantly altered in ALS."

The researchers will identify key RNA targets regulated by TDP-43 that may be abnormally changed in ALS. The work will provide valuable insight into how TDP-43 malfunction may contribute to neurodegeneration. Since the majority of ALS cases show TDP-43-associated pathology, the research could lead to a better understanding of what causes neurodegeneration in ALS patients.

RESOURCES

- ALS mutations database <http://alsod.iop.kcl.ac.uk/index.aspx>
- 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=3475>
- SOD1 mutant mice, Jackson Laboratory mouse models <http://jaxmice.jax.org/index.html>
- Jackson Laboratories ALS Mouse Repository http://www.jax.org/news/archives/2010/als_repository.html
- Control and SOD1 fibroblasts <http://www.alzforum.org/>
- Control and SOD1 fibroblasts http://ccr.coriell.org/Sections/Search/Advanced_Search.aspx?PgId=175&ct=C&result=y&coll=ND
- ALS Untangled <http://www.wfnals.org/alsu.html>
- ALS Association Research Webinars <http://www.alsa.org/research/research-webinars.html>

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.

August 2011 Ubiquilin-2 discovery; September 2011 C9orf72 discovery

2004

2005

2006

2007

2008

2009

2010

2011

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.

Journal News

The Role of Astrocytes in ALS

Several publications in the last few months highlight the importance of astrocytes in the disease process. Earlier studies using transgenic modeling and removing the mutant SOD1 from the astrocytes resulted in an increase in survival. This provided compelling evidence that a therapeutic approach targeting the astrocytes would have potential benefit in patients. The current studies address the question further by observing the effect of human astrocytes on motor neurons and identifying the toxicity induced by these cells.

In a study lead by Dr. Amanda Phillips in Dr. Brian Kaspar's laboratory at Nationwide Children's Research Institute (NCRI) in Columbus, Ohio and published in *Nature Biotechnology*, investigators used autopsy tissue from ALS patients with and without the SOD1 mutation to generate astrocytes in culture. They showed that these astrocytes were toxic to the surrounding motor neurons in co-cultures. Furthermore, lowering levels of SOD1 in the astrocytes obtained from individuals who died from both familial and sporadic ALS decreased the toxicity to the motor neurons providing further support that SOD1 may be important in mediating the disease not only in those cases with mutations in the gene but also in people with sporadic ALS.

<http://www.ncbi.nlm.nih.gov/pubmed/21832997>

The *in vitro* finding was also demonstrated *in vivo* by Dr. Nicholas Maragakis and colleagues and reported in *PLoS One*. The investigators transplanted a type of stem cell known as a glial restricted precursor (GRP) that contained the familial ALS version of SOD1 into the spinal cords of healthy rats. The GRP cells can only differentiate efficiently into astrocytes, not motor neurons or microglia, so the researchers knew that any damage to the normal rat's motor neurons would be from the mutated astrocytes. The rats that received the GRP cells with the SOD1 mutation showed motor neuron damage three months after transplantation. The motor neurons near the transplant site had aggregates of small molecules that were a cellular response to a toxic threat. As well, the rats with the mutated SOD1 astrocytes lost forelimb strength and respiratory function more than control rats.

<http://www.ncbi.nlm.nih.gov/pubmed/21998733>

The basis of the astrocyte toxicity is not clearly understood but is the subject of investigation worldwide. Investigators in Dr. Pamela Shaw's Laboratory at the University of Sheffield, United Kingdom and led by Dr. Laura Ferraiuolo, reported in *Brain* that toxicity may be due to two key mechanisms, metabolic dysregulation and/or dysregulation of the ratio of pro-nerve growth factor to mature nerve growth factor. They identified these key pathways using gene expression profiling of spinal cord astrocytes from presymptomatic transgenic mice expressing mutant superoxide dismutase and followed up their findings with *in vitro* studies, co-culturing astrocytes from these mice with healthy motor neurons. Furthermore, they validated their studies using samples from ALS patients.

<http://www.ncbi.nlm.nih.gov/pubmed/21908873>

Journal of Clinical Investigation

Investigators led by Dr. Luis Barbeito, Montevideo, Uruguay, demonstrated this week in the *Proceedings of the National Academy of Sciences* that astrocytes with an aberrant phenotype could be isolated from primary spinal cord cultures of symptomatic SOD1 rats. These astrocytes secreted soluble factors inducing motor neuron death. The study highlights that a subset of astrocytes arising during disease progression with unprecedented proliferative and neurotoxic capacity may be a potential target for slowing disease progression.

<http://www.ncbi.nlm.nih.gov/pubmed/22010221>

What is the role of TDP-43 and FUS in ALS?

Two proteins with similar structure and function TDP-43 and FUS linked to familial ALS with and without frontotemporal dementia are thought to be involved in the disease, either by causing some new toxic property or by a loss of their normal function. To identify the potential mechanism, investigators led by Dr. Brian McCabe at Columbia University Medical Center used the Drosophila-fly model. They demonstrated that these two proteins work in tandem to support the long-term survival of motor neurons. The findings were published in the September 1, 2011 online edition of the *Journal of Clinical Investigation*.

<http://www.ncbi.nlm.nih.gov/pubmed/21881207>

Coffee and ALS

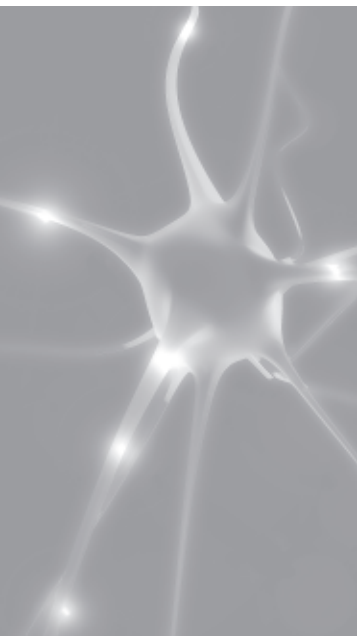
The relation between coffee intake and risk of amyotrophic lateral sclerosis (ALS) was investigated in 377 newly diagnosed ALS patients from four Italian population-based registries in the European ALS Consortium (EURALS Group) (2007–2010). While assessing the independent role of trauma and adjusting for possible confounders, including age, sex, physical activity, alcohol drinking, smoking, and coffee intake, investigators led by Dr. Ettore Beghi and published in *American Journal of Epidemiology* detected an inverse association between coffee consumption and risk of ALS.

<http://www.ncbi.nlm.nih.gov/pubmed/21946385>

Detecting Early Changes in ALS

Using magnetic resonance spectroscopy (MRS), a non-invasive method for *in vivo* measurement of tissue metabolites, and published in *Neurology*, investigators in Dr. Michael Benatar's laboratory showed that changes in neurometabolite ratios in the cervical spinal cord are evident in asymptomatic SOD1 people in advance of the onset of symptoms and clinical or electromyographic signs of the disease. These changes primarily reflect a reduction in ratios of N-acetylaspartate to creatine and N-acetylaspartate to myo-inositol. These studies are encouraging as identifying measures for early detection of ALS is crucial for improved treatment of the disease.

<http://www.ncbi.nlm.nih.gov/pubmed/21940617>



The ALS Association National Office
1275 K. Street NW, Suite 1050
Washington, DC 20005
www.alsa.org