RESEARCH ALS TODAY

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INSIDE

Neurofilaments as Potential Biomarkers for ALS

Kevin Boylan, M.D.

Department of Neurology, Mayo Clinic, Jacksonville, Florida and Medical Director, The ALS Association Certified Center

Wide variation in the rate of progression and survival are recognized characteristics of amyotrophic lateral sclerosis (ALS), and estimation of prognosis and monitoring of clinical progression are of central importance in patient care and clinical research. Assessment of the progression of ALS presently relies on clinical outcome measures, such as functional rating scales and tests of muscle strength, because there are no direct tests of nerve damage that have proven reliability. There is a strong need for disease biomarkers in ALS that demonstrate greater sensitivity to disease progression and survival than those presently available.

Development of tests that can identify and track molecular indicators of disease activity, or biomarkers, in cerebrospinal fluid (CSF) and peripheral blood in ALS has been a focus of recent research at several centers worldwide, but no biomarkers of this kind have as yet been fully validated for use in clinical practice or clinical trials. Tests of this kind could offer more sensitive and efficient ways to measure progression of ALS, provide more direct ways to evaluate the effectiveness of new medications and potentially facilitate the process of establishing the diagnosis of ALS.

Candidate markers of nerve cell damage in ALS include biomarkers linked to degeneration of motor neurons, such as the subunits of neurofilaments, major structural components of neurons. Three major protein subunits of neurofilaments are designated heavy (NF-H), medium (NF-M) and light (NF-L) subunits on the basis of molecular weight. These neurofilament subunits are heavily concentrated in large motor axons, and neurofilament subunits are expected to be released in significant quantities following axonal damage or degeneration. Several research groups reported that concentrations of NF-H and NF-L are increased in CSF in ALS compared with healthy subjects and patients with other neurological disorders, supporting the concept that elevated pNF-H and NF-L concentrations may be useful biomarkers in ALS. Of particular importance were early reports that the concentrations of pNF-H and NF-L in CSF may correlate with the rate of disease progression and survival in ALS.

Interest in NF-H as a potential ALS biomarker is based in part on structural characteristics of the protein that confer enhanced stability in CSF and

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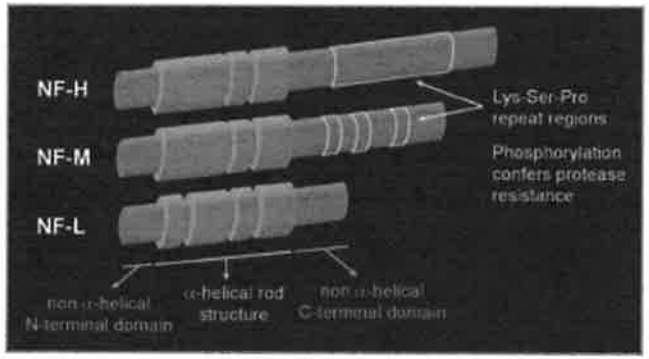
NEUROFILAMENT SUBUNITS



Neurofilament subunits are rod-like proteins *found in nerve cells. Of the three neurofilament subunits, NF-H contains the longest series of lysine-serine-proline tandem repeats. In motor axons these repeats tend to be modified by attachment of phosphorous, referred to as phosphorylation. Phosphorlyation confers resistance to normal breakdown processes and appears to make pNF-H more stable in peripheral blood and CSF, a useful property in clinical testing. Degeneration of motor neurons releases pNF-H into the CSF and peripheral blood stream, where its concentration can be measured.*

Kevin Boylan, M.D.

ALS Association
TREAT ALS
Translational Research Advancing Therapy for ALS

Increased Efforts to Find Biomarkers and Improve Clinical Trials

This year began with the disappointing negative outcome of the much awaited trial EMPOWER. With that news, however, comes even more determination to commit the much needed talents and resources to finding treatments for ALS. It highlights the need to really understand the disease process and identify those pathways that are most important in the disease and most likely when modulated to have a positive effect in patients. There is a critical need for the development of pharmacodynamic markers that will confirm whether a treatment approach has affected the pathway of interest in patients, and biomarkers are crucial for the selection of the appropriate patients for clinical trials. Dr. Kevin Boylan highlights some of the promise looking at neurofilaments and how they may be used for selection of patients in clinical trials. The ALS Association is committed to working with the community, both academia and industry to enable these advances. We are encouraged by the growing interest in the industry sector and the commitment of companies like Biogen to invest in drug development for ALS. Several promising trials are in the pipeline and are reviewed by Dr. Shefner in this edition.

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

We are pleased to recognize the talents of investigators such as Rosa Rademakers, Ph.D. and Bryan Traynor, M.D., Ph.D. through the Sheila Essey Award in partnership with the American Academy of Neurology. These investigators, together with their team and collaborators, have shifted the field of ALS into an exciting new era, and their findings have led to increased interest in ALS and frontotemporal dementia globally with articles arising from these findings published almost weekly.

The ALS Association is pleased to honor Rick Olney, M.D., who sadly lost his fight against ALS in 2012. Dr. Olney gave so much to the ALS community, not only as an ALS clinician and scientist but as a person living with the disease. We are pleased that Dr. James Berry is the recipient of this prestigious award.

As we look forward to the research accomplishment for 2013, we reflect on the needs of those people living with ALS and feel even more committed to improving their lives and finding treatments and a cure for the disease.

-Lucie Bruijn, Ph.D.

Rosa Rademakers, Ph.D. and Bryan Traynor, M.D., Ph.D. Receive the Sheila Essey Award

Each year, the American Academy of Neurology and The ALS Association present this award in memory of Sheila Essey, who battled ALS for 10 years and died from the disease in 2004. The Award is made possible through the generosity of the Essey Family Fund. Past recipients have utilized the funds to continue ALS research or to support promising young scientists in their research teams. This year The ALS Association is very pleased to recognize two individuals who will receive the Sheila Essey Award at The American Academy of Neurology Meeting in San Diego. Rosa Rademakers, Ph.D., and Bryan Traynor, M.D., Ph.D., identified the mutation on Chromosome 9, a landmark finding in ALS. The C9orf72 mutation is the most common known cause of familial and sporadic ALS and FTD and has transformed our understanding of these diseases. It is the most significant genetic discovery since the identification of mutations in SOD1. Unlike SOD1, mutations in C9orf72 are associated with TDP-43 inclusions pathologically, which is the dominant pathway implicated in 95% of ALS cases and approximately 50% of FTD. Investigators worldwide have been committed to identifying the gene alteration, and until now it had remained elusive. "Since all routine methods of genetic analysis had failed to find the genetic defect in this region, we suspected the defect could be a rare DNA repeat expansion," said lead investigator Mariely DeJesus-Hernandez from the Mayo Clinic-led research team. This team found an area of DNA that in healthy individuals is normally repeated only two to 23 times, but in ALS or FTD patients is repeated 700 to 1,600 times. These changes were found in almost 12 percent of familial FTD and more than 23 percent of familial ALS samples studied at Mayo Clinic.

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RESEARCH WEBINAR SERIES

March 26, 2013 / 4 - 5 p.m. EDT
Telehealth Program for the Integrated Care of Patients with ALS (telePALS)
James Berry, M.D., MGH, Boston, MA, Recipient of the Richard Olney Clinical Research Fellowship Award

April 23, 2013 / 4 - 5 p.m. EDT
A Novel Immunosuppressant Regimen for the Treatment of ALS
Jonathan Glass, M.D., Emory University, Atlanta, GA

May 21, 2013 / 4 - 5 p.m. EDT
A Multi-Center Randomized Controlled Study of the NeuRx Diaphragm Pacing System (DPS) in Participants with ALS
Kristen Gruis, M.D., SUNY Upstate Medical University, NY

June 25, 2013 / 4 - 5 p.m. EDT
MusK Agonists as a Treatment for ALS
Steve Burden, Ph.D., Skirball Institute, New York University, NY

July 25, 2013 / 4 - 5 p.m. EDT
ALS Research Overview
Lucie Bruijn, Ph.D., Chief Scientist, The ALS Association

Rademakers, Ph.D., and Traynor M.D., Ph.D.—Sheila Essey Award Recipients

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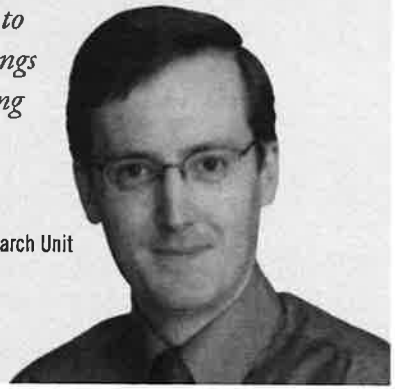
Rosa Rademakers' research ranges across Alzheimer's disease (AD), frontotemporal dementia (FTD) and more recently amyotrophic lateral sclerosis (ALS). Her expertise is grounded in genetics but extends into the fields of cellular biology and molecular pathology. She now heads one of the most successful neurodegeneration research laboratories in the world. Her contribution to ALS research began by exploring the molecular links to FTD through the contribution of common genes such as *progranulin (PGRN)*, but she has increasingly focused on genes that when mutated cause a predominantly motor neuron disease phenotype, including *Dynactin (DCTN1), TAR DNA Binding Protein (TARDBP), Fused in Sarcoma (FUS) and ataxin 2 (ATXN2)*. She has described a large number of novel mutations and the effects of mutation on protein localization in cellular models. She has also significantly contributed to pathological studies on transgenic mouse and human post-mortem tissues as well as correlating genetic defects to clinical and imaging phenotypes. This breadth of research activity is unusual for any biomedical scientist and reflects Dr. Rademakers' wide-ranging interest and abilities. Her most significant contribution however relates to the Chromosome 9p locus in ALS and FTD. Although linkage to Chromosome 9 had been reported in 2006 in a handful of families with ALS and FTD the causative gene mutation remained elusive despite the best efforts of many leading laboratories. She has followed this with a number of reports characterizing the clinical, imaging and pathological features of this cohort. Dr. Rademakers' findings suggest that the disease mechanism underlying C9orf72 mutations is of nuclear retention of RNA containing the hexanucleotide repeat and sequestration of proteins that regulate RNA processing that initiates neurodegeneration.

*"**I am truly honored** to receive the prestigious 2013 Sheila Essey Award for ALS research. This prize represents welcome recognition of my team and my collaborators, without whom this project would not have been successful. We continue to work together to understand the mechanisms by which the C9orf72 repeat expansion leads to neurodegeneration and to translate these findings back to the bedside to help the patients suffering from this terrible disease."*

Bryan Traynor, M.D., Ph.D.
Investigator and Chief of the Neuromuscular Diseases Research Unit
Laboratory of Neurogenetics, National Institute on Aging,
National Institutes of Health
Bethesda, MD



*"**I am very excited** that the work of my research group on the Chromosome 9p discovery is being recognized with this prestigious award! It's a real honor."*

Rosa Rademakers, Ph.D.
Associate Professor of
Neuroscience, College of Medicine,
Mayo Clinic, Jacksonville, FL

Bryan J. Traynor is a neurologist and an investigator at the National Institute on Aging, where he has been the Chief of the Neuromuscular Diseases Research Unit at the Laboratory of Neurogenetics since 2009. His laboratory is focused on unraveling the genetic etiology of ALS (also known as Lou Gehrig's Disease) and other neuromuscular disorders. Dr. Traynor is best known for his work aimed at understanding the genetic etiology of ALS. His laboratory published the first genome-wide association study of ALS (2007); was the first to identify an association signal for ALS on the short arm of Chromosome 9 in the Finnish founder population (2010); and discovered that mutations in the VCP gene are responsible for a significant fraction of familial ALS (2010). In 2011, he led the international consortium that identified the pathogenic hexanucleotide repeat expansion in the C9orf72 gene. Dr. Traynor has served as scientific reviewer for The ALS Association and as advisor to many of the research initiatives.

"I am extremely excited that these investigators have been recognized with the Sheila Essey Award for this pivotal finding and their dedication to ALS research," commented ALS Association Chief Scientist Lucie Bruijn, Ph.D. "The identification of C9orf72 will significantly impact the field as we begin to understand more about the consequence of these changes to the disease process, aid our understanding of FTD and ALS, potentially provide a diagnostic tool, and enable the development of new therapeutic approaches."

ALS Clinical Trial Update

Jeremy Shefner, M.D., Ph.D.

The clinical trial landscape is replete of agents aimed at a variety of targets, all with the goal of slowing ALS disease progression or enhancing function in ALS patients. In the last several months, three trials have been completed, with all failing to meet their primary endpoint. Despite this, valuable lessons have been learned, and many studies are either enrolling subjects or upcoming.

Ceftriaxone

In July 2012, the phase 3 study of ceftriaxone in ALS was halted by NIH. Ceftriaxone was identified as a potentially useful agent in ALS through a large screening program in which hundreds of drugs were evaluated using 17 different assays of neurodegeneration. 504 subjects were randomized either to four grams or placebo. The study was intended to be completed when the last enrolled subject completed 12 months of active treatment; the study was halted approximately six months prior to the targeted end date because of a potential for increased risk in the placebo group, as well as a low likelihood that the results would show a statistically significant benefit.

Nonetheless, trends in the data provided encouragement for the future. Both survival and function were approximately 10 percent improved in patients on ceftriaxone. These trends suggest that further study of agents intended to modulate glutamate toxicity are warranted.

Dexpramipexole

In January 2013, Biogen Idec announced that its study of dexpramipexole in ALS had failed to demonstrate any beneficial effect. This was a blow to both patients and the entire ALS community, as the study followed a phase 2 trial that was extremely encouraging. In that study, encouraging dose-dependent trends were noted in both ALSFRS-R and survival. A post hoc joint rank test combining survival and function was statistically significant.

Based on these promising preliminary results, 953 subjects in North America and Europe were enrolled in a phase 3 trial. Subjects were enrolled to receive either 300 mg of dexpramipexole or placebo daily for at least 12 months. Overall, dexpramipexole was well tolerated; however the results did not suggest a benefit of this drug in either function or survival.

As noted previously, this result was a grave disappointment for all in the ALS community. However, the study energized both patients and investigators, such that enrollment of this very large study was accomplished in record time. The rapid enrollment and excellent conduct of this study are aspects that can be translated into more rapid evaluation of promising agents in the future.

NP001

In October 2012, Neuraltus Pharmaceuticals reported the results of a phase 2 study in which 136 patients were randomized to receive either placebo, or 1 mg/kg or 2 mg/kg intravenous infusion of NP001 over a period of six months, followed by a six-month follow-up period. While the primary analysis revealed no significant differences between groups, encouraging trends raised the possibility that a phase 3 trial might show meaningful benefit.

Ongoing and Upcoming Trials

Two large trials are ongoing that provide hope for ALS patients. Though the intended mechanisms of the agents being tested are quite disparate, they both have the goal of improving or maintaining function in ALS, rather than addressing underlying mechanisms of disease.

Tirasemtiv, being developed by Cytokinetics, is a fast skeletal muscle activator that improves the efficiency of muscle contraction. By increasing the sensitivity of the muscle contractile apparatus to calcium, the force produced by muscle contracting at submaximal stimulation frequencies is enhanced. In three early phase studies in ALS patients, encouraging results were obtained, with side effects primarily limited to dizziness.

An ongoing study will enroll approximately 400 patients in North America and Europe, evaluating functional effects of tirasemtiv in patients over a three-month period. The primary outcome measure is ALSFRS-R, but multiple measures of neuromuscular endurance and strength will also be obtained. While the mechanism of action of tirasemtiv may not affect the underlying progression of ALS, functional preservation or improvement would be extremely valuable to patients striving to maintain independence in their daily lives.

Another ongoing study is sponsored by Glaxo Smith Kline, and is studying a monoclonal antibody, ozanezumab, which is intended to enhance the ability of nerve fibers to contact individual muscle fibers. A group of 300 patients will be randomized to receive either ozanezumab or placebo. Both survival and ALSFRS-R will be studied.

Jeremy Shefner, M.D., Ph.D. Co-Chair North East ALS Consortium

In April, a study will begin that will investigate whether diaphragmatic pacing preserves respiratory function and prolongs life in ALS. A prior study of diaphragm pacing (DPS) manufactured by Synapse Biomedical compared treated patients to a historical database of ALS patients and suggested a possible survival effect.

The planned study, funded jointly by The ALS Association, the Muscular Dystrophy Association, and Synapse, will evaluate the efficacy of DPS with patients randomized either to pacemaker insertion or receiving respiratory support. A number of measures of respiratory function will be assessed, with survival being the primary outcome.

Another study with the goal of functional improvement evaluates the potential of Nuedexta, a newly approved drug that improves emotional lability in ALS patients, to improve swallowing and speech in ALS. This study is supported by a number of clinical observations suggesting that these functions are improved when patients take Nuedexta for its intended purpose. The primary outcomes evaluate changes in swallowing and speech. The study, funded by The ALS Association TREAT ALS™ program, the NEALS TREAT ALS™ network, a donation made to the Neurology Clinical Trials Unit at Massachusetts General Hospital, and Avanir Pharmeceuticals,

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TIMELINE

1860
- ***1986:*** *French neurologist Jean-Martin Charcot identifies ALS*

1950
- ***50s:*** *DNA structure solved*
- ***50s:*** *Nerve growth factor (NFG) identified–protective, growth promoting factor for nerve cells*

1960
- ***1968:*** *SOD1 enzyme identified*

1970
- ***70s:*** *Programmed cell death in motor neurons demonstrated*

1980
- ***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
- ***1990:*** *Congress declares the 1990s the "Decade of the Brain"*
- ***1990:*** *Growth factor CNTF is found to increase survival of motor neurons*

1991
- ***1991:*** *Researchers link familial ALS to Chromosome 21*

1992
- *The ALS Association begins workshops*
- *Glutamate transporter shown to be defective in ALS*
- *Growth factor BDNF found to increase survival of motor neurons*

ALS Clinical Trial Update Continued from page 4

Full Study Name	Principal Investigator	Type of Clinical Trial	Expected Number of Participants	# of Sites	Currently Enrolling?
A Phase IIb, Multi-National, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability and Efficacy of CK-2017357 in Patients with ALS (BENEFIT-ALS)	Cytokinetics	Interventional	400	70-76	Yes
Phase II/III Randomized, Placebo-controlled Trial of Arimoclomol in SOD1 Positive Familial Amyotrophic Lateral Sclerosis	Michael Benatar (University of Miami)	Interventional	80	2	Yes
Trial of Resistance and Endurance Exercise in ALS	Nicholas Maragakis (Johns Hopkins University)	Interventional	60	4	Yes
BrainGate2: Feasibility Study of an Intracortical Neural Interface System for Persons with Tetraplegia	Leigh Hochberg (Massachusetts General Hospital)	Interventional	15	2	Yes
A Dose-Escalation Safety Trial for Intrathecal Autologous Mesenchymal Stem Cell Therapy in Amyotrophic Lateral Sclerosis	Anthony Windebank (Mayo Clinic)	Interventional	25	1	Yes
HDE Post-Approval Study (PAS) of NeuRx DPS for ALS	Robert Miller (California Pacific Medical Center)	Interventional	60	1	Yes
Phase I/II Study of SOD1 Inhibition by Pyrimethamine in Familial ALS	Dale Lange (Weill Medical College of Cornell University)	Interventional	40	4	Yes
Randomized Double-Blind Placebo-Controlled Cross-Over Study of Incobotulinum Toxin A (Xeomin®) for Troublesome Sialorrhea in Parkinson's Disease and ALS	MERZ Pharmaceuticals	Interventional	20	1	Yes
Trial of High-fat/High-Calorie Diet versus Optimal Nutrition in Amyotrophic Lateral Sclerosis	Anne-Marie Wills (Massachusetts General Hospital)	Interventional	35	12	No
Phase 2 Selection Trial of High Dosage Creatine and Two Dosages of Tamoxifen in ALS	Nazam Atassi (Massachusetts General Hospital)	Interventional	104	14	No
An Open-Label, Safety and Tolerability, Study Evaluating KNS-760704 in Patients with Amyotrophic Lateral Sclerosis (ALS)	Merit Cudkowicz (Massachusetts General Hospital)	Interventional	90	19	No
A Clinical Demonstration of EEG Brain-computer Interface for ALS Patients	Robert Ruff (VA Medical Center, Cleveland)	Interventional	25	5	No
A Multi-Center Controlled Screening Trial of Safety and Efficacy of Rasagiline in Subjects with Amyotrophic Lateral Sclerosis	Yunxia Wang (University of Kansas)	Interventional	30	10	No

Full Study Name	Principal Investigator	Type of Clinical Trial	Expected Number of Participants	# of Sites	Currently Enrolling?
The Pre-Familial Amyotrophic Lateral Sclerosis (Pre-fALS) Study	Michael Benatar (University of Miami)	Observational	300	1	Yes
A Longitudinal Study of Amyotrophic Lateral Sclerosis (ALS) Biomarkers	Merit Cudkowicz (Massachusetts General Hospital)	Observational	250	6	Yes
Non Invasive Examination of the Work of Breathing in Patients With Amyotrophic Lateral Sclerosis	Terry Heiman-Patterson (Drexel University)	Observational	40	1	Yes
Non Invasive Measurement of GI Motility in Patients With ALS	Terry Heiman-Patterson (Drexel University)	Observational	60	1	Yes
Quantitative Measurement of Nutritional Substrate Utilization in Patients With Amyotrophic Lateral Sclerosis	Terry Heiman-Patterson (Drexel University)	Observational	90	1	Yes
EEG-Based Brain-Computer Interface Project for Individuals With ALS	Terry Heiman-Patterson (Drexel University)	Observational	50	1	Yes
Skin Biopsies for Research in Motor Neuron Diseases	James Berry (Massachusetts General Hospital)	Observational	100	1	Yes
Analysis of Immune Activation in the Peripheral Blood of Patients with ALS	James Berry (Massachusetts General Hospital)	Observational	200	1	Yes
Assessing Activation of Brain Microglia in ALS with Simultaneous MR-PET	Nazam Atassi (Massachusetts General Hospital)	Observational	25	1	Yes
DNA, Blood and Skin Cell Repository for Research on ALS and Related Neurodegenerative Disorders at Mayo Clinic Florida	Kevin Boylan (Mayo Clinic Florida)	Observational	160	1	Yes
Family Studies in Neuromuscular Disorders	Robert Brown (University of Massachusetts, Worcester)	Observational	6,000	1	Yes
Identification of Genes Causing Familial ALS or Increasing Risk for Sporadic ALS and ALS With Frontotemporal Dementia and Understanding Disease Mechanism	Teepu Siddique (Northwestern University)	Observational	15,000	1	Yes
A Multi-Center Study for the Validation of ALS Biomarkers	Merit Cudkowicz (Massachusetts General Hospital)	Observational	650	31	No
Validation of a New Device to Measure Neuromuscular Disease Progression	Patricia Andres (Massachusetts General Hospital)	Observational	70	5	No

TIMELINE cont.

1993
- *SOD1 gene mutation (chromosome 21) discovered in familial ALS*
- *Trials using glutamate blocker riluzole begin*

1994
- *Animal studies combining CNTF and BDNF demonstrate decreased motor neuron loss*
- *GDNF rescues degenerating motor neurons during development in an **in vitro** experiment*

1995
- *FDA approves riluzole*

1996
- *Toxic properties of the SOD1 enzyme discovered and linked to familial ALS*

1997

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

1999

2000
- *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*

2001
- *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/NINDS collaborative effort begins screening drugs*

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

Neurofilaments as Potential Biomarkers for ALS

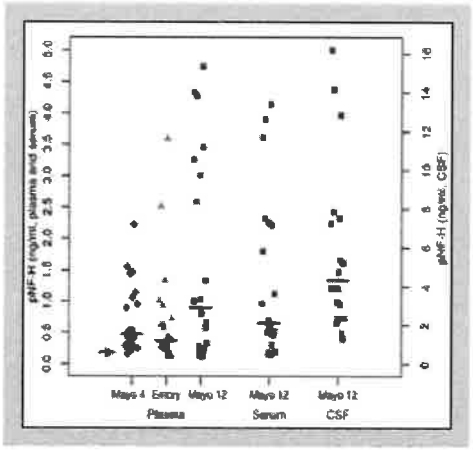
Continued from page 1

peripheral blood. Dr. Gerry Shaw's laboratory at the University of Florida developed an enzyme-linked immunosorbent assay (ELISA) to detect pNF-H and showed that pNF-H can be readily detected in not only CSF, but also blood of animal models of central nervous system injury and disease, as well as in patients with neurological conditions expected to result in axonal loss such as cerebral hemorrhage. These studies included work in collaboration with Dr. David Borchelt at University of Florida to show that average pNF-H levels in the blood of the G93A SOD1 mouse model of ALS increase progressively from early- through late-stage disease.

Given difficulties inherent in collecting CSF compared to peripheral blood for clinical testing and research, we have conducted a series of studies with Dr. Shaw to evaluate pNF-H levels in plasma and serum in comparison with CSF in ALS patients using a new monoclonal antibody-based ELISA developed by Dr. Shaw. Clinical outcomes were assessed using the ALS functional rating scale revised version (ALSFRS-R) and survival. In our initial pilot study of pNF-H using this assay we measured pNF-H levels in plasma monthly for four months in ALS patients compared with similarly aged healthy control individuals in order to see whether pNF-H levels were increased in peripheral blood in ALS, and whether the levels changed significantly over time. We found that the median plasma pNF-H level in ALS patients was 2.8-fold higher than in controls, and that while some of the ALS patients had higher pNF-H levels than others, for a given patient pNF-H levels were relatively stable over the four-month follow-up interval.

Based on these results we next looked at pNF-H levels in plasma, serum and CSF in ALS patients whom we followed clinically for up to 12 months after sample collection at Mayo Clinic. We also studied associations between pNF-H concentration in plasma and survival in two independently collected ALS cohorts at Mayo Clinic Florida and an ALS cohort studied by Dr. Jonathan Glass at Emory University, and compared data from a similar analysis of pNF-H levels in serum and CSF in a subgroup of these patients. Similar to earlier findings, higher pNF-H levels in plasma, serum and CSF were associated with faster decline in ALSFRS-R at follow-up month four, and also at month 12 for CSF pNF-H levels. In addition, higher serum and plasma pNF-H levels were associated with shorter survival from the point of sample collection, although evidence was weaker for CSF. A key observation in the interpretation of these results is that in our studies there was not a perfect correlation between pNF-H levels and clinical outcome, indicating that while pNF-H levels appear to offer some prognostic information with current test methods, there likely are technical factors involving the ELISA, which require additional refinement in order to improve its reliability. There also may be clinical characteristics of ALS that need to be considered in the interpretation of pNF-H findings in each patient.

Recent findings are encouraging but much remains to be done before pNF-H can be considered a standard test in patient care and research. Studies to date have involved relatively small numbers of patients, and there is limited information on the extent to which pNF-H levels may change over time in peripheral blood and CSF in ALS; larger scale longitudinal studies are needed. Further research also is needed to characterize the time course of pNF-H release and breakdown in peripheral blood and CSF in ALS in order to evaluate pNF-H as a potential indicator of response to treatment. There is also a need for standardization of pNF-H ELISA methodology for larger-scale applications. These challenges are surmountable, and studies to date provide a sound rationale for continued efforts to establish pNF-H as a biomarker for ALS.



Distributions of pNF-H concentrations in plasma, serum and CSF in ALS patients. *Blue squares represent patients in the Mayo Clinic 12-month follow-up cohort (Mayo 12; plasma, serum and CSF), green triangles represent patients in the Emory University cohort (Emory; plasma only) and black diamonds represent patients in the Mayo Clinic four-month follow-up cohort (Mayo 4; plasma only). Black horizontal lines represent the median pNF-H level for the given cohort. The left vertical axis scale reflects pNF-H levels in plasma and serum and the right vertical axis scale represents pNF-H levels in CSF. The median level of pNF-H in plasma in healthy control subjects in our initial pilot study, 0.17 ng/ml, is shown for comparison by the orange arrow. pNF-H levels in plasma, serum and CSF in ALS cover a wide range, and plasma levels in some patients are in the same range as those found in healthy people. While pNF-H by itself is clearly not a diagnostic marker for ALS, the concentration has demonstrated an association with the rate of disease progression.*

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TIMELINE cont.

2003

- 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
- 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

2004

- Study implicates smoking as likely risk factor in sporadic ALS
- Study releases evidence that mitochondrial malfunction may play an important role in ALS
- Study funded by The ALS Association to find biomarkers in cerebrospinal fluid and blood

2005

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

2006

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

2007

- 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
- First genome screening data published based on NINDS ALS Repository

ALS Clinical Trial Update

Continued from page 4

will enroll 60 patients and is expected to start in April 2013.

Mexiletine is a currently available drug used primary for cardiac rhythm disturbances. It was also demonstrated to have a survival effect in the SOD1 transgenic mouse model. Laboratory studies suggest that mexelitine is neuroprotective via a number of possible mechanisms. For these reasons, a study to evaluate to safety and tolerability of mexelitine in ALS will be performed on 60 patients with ALS, with patients receiving either placebo, 300 mg mexelitine daily, or 900 mg daily for 16 weeks. This study is funded by the ALS Therapy Alliance and a donation made to the Neurology Clinical Trials Unit at Massachusetts General Hospital.

As the above summary makes clear, ALS clinical research remains robust and exciting, despite recent setbacks. Other promising studies are also being planned. However, beyond a continued effort to bring potential treatment modalities to trial, it is clear that a renewed effort is necessary to improve the speed at which drugs come to trial. Just as important is the investigation of potential markers of disease progression, or indicators of activity of experimental agents against their intended target. By developing such markers, early phase trials can be shortened, and a more rational framework can be developed for making decisions on promising drugs.

For more details about ongoing clinical trials http://www.alsconsortium.org/search.php

Richard Olney AAN/ALS Association Clinician Scientist Development Award

The ALS Association and the American Academy of Neurology (AAN) are pleased to announce that James Berry, M.D., M.P.H., from the Department of Neurology, Massachusetts General Hospital, Boston, Mass., is this year's recipient for the Richard Olney AAN/ALS Association Clinician Scientist Development Award, which is a part of The Association's research program TREAT ALS (**T**ranslational **Re**search **A**dvancing **T**herapy for ALS). The purpose of the award is to recruit talented and promising young clinicians who propose innovative clinical research and to foster their development to make significant contributions to ALS clinical research. The award is named in memory of Richard K. Olney, M.D., a leading ALS neurologist and researcher. Dr. Olney, who died of ALS in 2012, was the director of the ALS Treatment and Research Center at the University of California, San Francisco.

For his Fellowship research, Dr. Berry will lead a longitudinal study to characterize immune system changes that may be used as a biomarker in ALS.

Biomarkers, measurable changes which can be correlated with disease and response to therapy, are a key unmet need in ALS. Such biomarkers would potentially allow shorter and smaller clinical trials, since they may be a more sensitive and rapid read-out of therapeutic response than clinical measures such as daily function or survival. Dr. Berry has worked under the guidance of Howard Weiner, M.D., of Brigham and Women's Hospital in Boston, as part of a team that has identified changes in pro-inflammatory monocytes as a potential biomarker for ALS progression. The Fellowship Award will allow Dr. Berry to further this research through both laboratory and clinical work.

James Berry, M.D., M.P.H.

Preliminary results have shown that monocytes, which are a type of immune cell, are activated in the periphery and recruited into the central nervous system in people with ALS. Dr. Berry will be further characterizing these changes in the monocyte populations in peripheral blood taken from patients over the course of their disease. In the second half of his Fellowship, mentored by Merit Cudkowicz, M.D., of Mass General, Dr. Berry will develop clinical protocols for collection, preservation and storage of monocyte samples from peripheral blood, as well as strengthen his clinical research skills for ALS clinical trials.

"I am thrilled to be this year's recipient of the Richard Olney AAN/ALS Association Clinician Scientist Development Award," said Dr. Berry. "I believe that the discovery of reliable biomarkers will have a marked impact on our understanding of ALS as well as on future clinical trials. Our early work on monocytes has been very promising, and the prospects for the longitudinal project are extremely exciting."

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
www.taconic.com/wmspage.cfm?parm1=1661

SOD1 mutant rats, Jackson Laboratory mouse models
http://jaxmice.jax.org/index.html

ALS news
http://www.alzforum.org/

Control and SOD1 fibroblasts
http://ccr.coriell.org/Sections/Collections/CSCB/Default.aspx

ALS Untangled
http://www.wfnals.org/alsu.html

ALS Mouse repository
http://jaxmice.jax.org/neurobiology/als.html

TIMELINE *cont.*

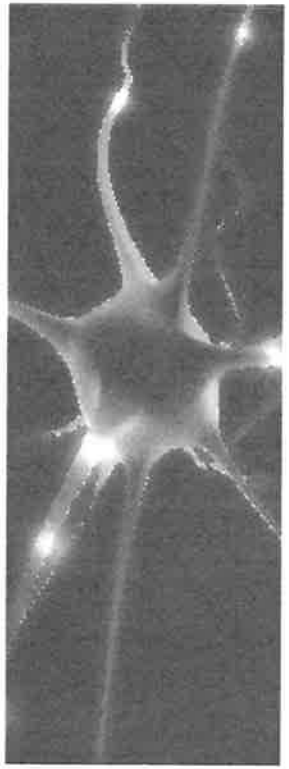
2008
- *Stem cells generated from ALS patients*
- *Discovery of DPP6 in two genome-wide association studies in ALS*
- *Mutations in TDP-43 linked to familial and sporadic ALS*
- *Induced Pluripotent Stem Cell Technology opens up new avenues for ALS*

2009
- *Identification of new gene linked to familial ALS, Fused in Sarcoma (FUS) on Chromosome 16*
- *FDA approval of SOD1 antisense and stem cell trials in U.S.*

2010
- *First patients enrolled for antisense and stem cell trials in U.S.*

2011
- *Ubiquilin-2 discovery; C9orf72 discovery*

2012
- *March: The ALS Association hosts 2nd Drug Discovery Workshop for ALS*
- *September: Researchers find genetic region influencing age at which people develop ALS*



ALS Association

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

JOURNAL NEWS

ALS-causing SOD1 Mutations Expose ER Protein Binding Site

Most SOD1 mutations expose a site on the protein that binds to an endoplasmic reticulum protein, derlin-1, according to new research, and mutant toxicity is correlated with derlin-1 binding.

Mutations in superoxide dismutase 1 (SOD1) are an important cause of familial ALS and provide the most widespread source of animal models for study of the disease. A major unanswered question in ALS is what the more than 100 different clinically identified SOD1 mutants have in common, and how they each cause the same disease. A leading hypothesis is that mutation induces a common conformational alteration, but the pathogenic consequences of that alteration remain unknown. Derlin-1 (degradation in endoplasmic reticulum protein 1) is a component of the ER-associated degradation (ERAD) machinery, which moves unfolded or misfolded proteins from the ER into the cytosol for degradation.

Fujisawa et al. have previously shown that three common ALS-causing SOD1 mutants (A4V, G85R and G93A) bind to the cytosolic region of derlin-1. In their latest study in the *Annals of Neurology*, they extend that work by showing the same phenomenon in 124 of the 132 known SOD1 mutants. Those SOD1 mutants that bound derlin-1 caused ER stress and increased motor neuron death in culture, while mutants that did not bind derlin-1 did not. More important, most of the SOD1 mutants that did not bind derlin-1 have not been confirmed as pathogenic mutations in ALS.

They created a monoclonal antibody to the binding site, and found that it precipitated SOD1 from 14 ALS patients with SOD1 mutations, but not three healthy controls, suggesting the antibody may have utility in ALS diagnosis. They also showed that serum starvation, which is known to cause ER stress, induced a mutant-like conformation of wild-type SOD1 protein, exposing the derlin-1 binding site.

Together, these results suggest that interruption of the ERAD response through derlin-1 binding may contribute to ALS caused by SOD1 mutations, and possibly non-SOD1 ALS as well. Further work will be needed to understand the extent of derlin-1 involvement in other models of ALS.
http://www.ncbi.nlm.nih.gov/pubmed/23280792

Carotenoids May Lower ALS Risk

Consumption of foods high in carotenoids may help prevent or delay onset of ALS, according to a new study from the lab of Alberto Ascherio and published in *Annals of Neurology*. Carotenoids are antioxidant and anti-inflammatory compounds found in orange-colored vegetables, such as carrots and sweet potatoes, as well as dark green vegetables, such as spinach. Among pooled prospective studies of over one million individuals, a total of 1,153 ALS deaths occurred. A greater intake of carotenoids was associated with a 25 percent reduced risk of ALS, while there was no effect for vitamin C. The differential effects of these antioxidants correlate with their differing water solubility, the authors note, although other factors may be at work.
http://www.ncbi.nlm.nih.gov/pubmed/23362045

Head Injury May Pose Short-term, but not Long-term, Risk of ALS

A retrospective study from Sweden of over 4000 ALS patients and 20,000 controls matched by gender and birth year found that severe head injury less than one year before diagnosis was associated with an almost four-fold increased risk of ALS. There was no association for severe or repeated head injury more than three years before diagnosis. The study was published in the journal *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, and was led by Tracy Peters. http://www.ncbi.nlm.nih.gov/pubmed/23286749

Different ALS Genes May Trigger Different Disease Processes

A new disease model of ALS, based on expression of TDP-43, has shown that mutant protein expression in astrocytes is harmful to the astrocytes themselves, but not to motor neurons. In contrast, overexpression of mutant SOD1 in astrocytes leads to death of motor neurons, even if the motor neurons do not bear the mutant gene. The research, conducted by Andrea Serio and led by Siddharthan Chandran of Edinburgh University, was carried out in cell culture using astrocytes and neurons derived from induced pluripotent stem cells, either with or without the TDP-43 mutation. The authors suggest that "reactive gliosis seen in ALS may not be simply a response to neuronal injury but a consequence or direct mutation-mediated astrocyte toxicity."

Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut W, Maniatis T, Shaw CE, Finkbeiner S, Chandran S. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. Proc Nat Acad Sci USA 2013; http://www.ncbi.nlm.nih.gov/pubmed/23401527

Neuronal Identity Can Be Changed After Birth

Neurons of one type can be transformed into another type even after birth, according to new research by Caroline Rouaux and Paola Arlotta of Harvard University, and published in *Nature Cell Biology*. The team showed that early post-natal neurons from the corpus callosum could be reprogrammed to become corticofugal neurons, a subtype that includes motor neurons, through expression of a neuronal transcription factor. The transformed neurons formed subtype-appropriate connections to targets below the cortex. http://www.ncbi.nlm.nih.gov/pubmed/23334497

Motor Neuron-Astrocyte Communication Profoundly Disrupted in ALS

Analysis of messenger RNA from motor neurons and astrocytes indicate that the SOD1 mutation alters each cell's gene expression profile in ways suggesting a mutually reinforced dysregulation of their normal communication. Mutant glia induced changes in cell membrane proteins and cell responses to stress or injury. TGF-beta-receptor type II expression in motor neurons increased early, before symptom onset in a mouse model, in parallel with an increase in reactive astrogliosis. The study was performed by Hemali Phatnani, Tom Maniatis and colleagues, and published in the *Proceedings of the National Academy of Sciences USA*.
http://www.ncbi.nlm.nih.gov/pubmed/23388633

C9orf72 Expansion is Translated into Insoluble Polypeptide

The expanded GGGGCC repeat of the C9orf72 gene is translated to form three different insoluble polypeptides, according to two new studies from the labs of Leonard Petrucelli of the Mayo Clinic in Jacksonville, Florida, and Dieter Edbauer of Ludwig-Maximilians University in Munich, Germany. The polypeptides are created when the expanded RNA from the gene is transcribed despite the absence of a conventional start codon, a process called "repeated-associated non-standard" or RAN translation. The phenomenon was first discovered in ataxias due to a CAG repeat. Both groups used antibodies to find polypeptides made from repetitions of two amino acids (glycine-proline, glycine-alanine, and glycine-arginine), consistent with the codons available in the RNA expansion. The dipeptide repeats were localized to neuronal cytoplasmic and intranuclear inclusions from ALS brain. Based on their association with affected parts of the brain, Mori et al. suggest these species are toxic.

Ash EPA, Bieniek KF, Gendron TF, Caulfield T, Lin WL, DeJesus-Hernandez M , van Blitterswijk MM, Jansen-West K, Paul JW 3rd, Rademakers R, Boylan KB, Dickson DW, Petrucelli L. Unconventional translation of C9orf72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron Feb. 20, 2013;77(4):639-46.
http://www.ncbi.nlm.nih.gov/pubmed/23415312

Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts C, Van Broeckhoven C, C Haass, Edbauer D. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide repeat proteins in FTLD/ALS. Science express. Published online Feb. 7, 2013
http://www.ncbi.nlm.nih.gov/pubmed/23393093