The central question of the “post-genome” era, “is how to translate genomic insights into therapeutics,” Dr. Austin said. 

In early March 2012, more than 120 researchers, drug developers, government officials, and others came together in Washington, D.C., for three days of intense discussions, all centered on one problem: How to advance drug discovery for ALS? “It was an exhausting meeting,” one participant said at the end, “but it was one of the best meetings of its kind I’ve been to.”

The magnitude of the challenge was lost on no one, especially as the meeting took place on an auspicious date, the 19th anniversary of the announcement of the discovery of the SOD1 mutation in familial ALS in March of 1993. “Nineteen years later, despite what we all hoped, no therapy has been developed from that discovery,” noted keynote speaker Christopher Austin, M.D., Scientific Director of the National Center for Translational Therapeutics (NCTT) at the National Institutes of Health.

And yet, the excitement about new discoveries, especially a new set of ALS genes, had most researchers optimistic that a way forward could be found. In this issue of Research ALS Today, we look at some of the challenges and the paths toward progress arising from the meeting.

The Challenges of CNS Drug Discovery

The central question of the “post-genome” era “is how to translate genomic insights into therapeutics,” Dr. Austin said. The problem exists for virtually all central nervous system conditions, and even for peripheral diseases. There are currently more than 6,000 rare or orphan diseases known, and genes for two-thirds of them have been identified. However, there are treatments for fewer than 250 of them.

The reasons are multiple, but a major one is the gulf between the work of basic researchers, who identify mechanisms and targets, and pharmaceutical companies, who alone have the resources to conduct large-scale...
New Discoveries and Collaborations Indicate Progress in ALS Research

There could not be a better time for ALS research than now with so many new discoveries and opportunities for researchers to work collaboratively with investigators worldwide to advance the field. This was evident from discussions among participants who attended the Drug Discovery Workshop last month. The highlights from the workshop are featured in this edition of Research ALS Today. ALS is becoming a much more tractable disease for the development of therapeutics with increased interest from the pharmaceutical companies and an increase in the number of potential targets to develop therapies. Several new collaborations were forged during the three days of presentations and discussions, including some between academia and industry. The challenges to develop therapies for ALS should not be underestimated and it certainly takes a team approach and significant resources to make it happen.

The ALS Association is pleased to honor the Sheila Essey recipient, Chris Shaw, M.D., whose contributions to ALS genetics has significantly impacted the current progress in the field, opening up several new areas of research. In addition, The ALS Association is proud to be partnering with the American Academy of Neurology to solicit applicants for the Richard Olney Clinician-Scientist Development Award, honoring an outstanding ALS clinician and scientist, who ironically succumbed to this devastating disease. We strongly believe that through our scientific and clinical fellowship awards, we can encourage a new generation of talented individuals to focus on ALS and make an impact in the field.

Several clinical trials for ALS are in progress, and we await the results with a hope that one or more will provide new treatments for ALS. In the meantime, there is still a great deal of research to be done to understand how the recently discovered genes lead to disease and build tools such as animal models to understand the sequence of events and identify new therapeutic targets. The progress being made in developing more relevant culture systems in a dish to model human disease is also very encouraging and provides an important tool for the screening of novel compounds.

-Lucie Bruijn, Ph.D.

Christopher Shaw, MBChB, M.D., FRACP, from the Institute of Psychiatry (IoP) at King’s College London, University of London, U.K., has been awarded the 2012 Sheila Essey Award for research into amyotrophic lateral sclerosis (ALS) by the American Academy of Neurology and The ALS Association. The Award recognizes individuals who have made significant research contributions in the search for the cause, prevention of, and cure for ALS.

Dr. Shaw is an accomplished molecular cell biologist as well as an outstanding clinician scientist, who actively contributes to clinical research at the King’s MND Care and Research Centre, London, and through his leadership of the European Familial ALS (FALS) Group. He has a growing national and international reputation, which has led to the development of collaborative genetics studies in Europe. He has built productive and friendly collaborations with outstanding scientists in Europe and North America, including many who have been awarded the Sheila Essey Award.

Dr. Shaw’s group was the first to report in the journal Science that mutations in the genes encoding two RNA-binding proteins, TDP-43 and FUS, can cause ALS/MND.

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Continued on page 3
and propagate neurodegeneration can we develop drugs that really alter the course of this terrible disease," Dr. Shaw said. He is currently the director of the Maurice Wohl Clinical Neuroscience Institute at the Institute of Psychiatry at King's College.

Dr. Shaw trained as a clinical neurologist in New Zealand and came to Cambridge (UK) on a Welcome Trust Fellowship to learn neurobiology and genetics. He completed a research doctorate on molecular signaling during axonal-glial interactions and joined the Institute of Psychiatry to work with Professor Nigel Leigh in the motor nerve clinic, where he continues to be an active clinician. With the support of Professors Leigh, Ammar Al-Chalabi, Christopher Miller and other colleagues, he has established one of the world’s leading ALS genetic and cell biology laboratories. He has spent 12 years working as a consultant neurologist and neurogeneticist at King’s College and has established the first Neurogenetic Clinic and Familial MND Clinics in the Southeast of England. He is currently the head of Department of Clinical Neuroscience, integrating clinical and research strategies.

Dr. Shaw’s early work focused on Cu/Zn Superoxide dismutase (SOD1) and with the ability to collect a large number of DNA samples, his team described many novel SOD1 mutations and explored their effect on SOD1 biochemistry. He was among the first to describe the molecular pathology and compare it to sporadic ALS.

"His outstanding contributions to the genetics of ALS mark a significant time in ALS research..."
—Lucie Bruijn, Ph.D.

Working with Dr. Peter Andersen and other ALS clinician-scientists, he identified a common founder for the D90A mutation, which arose approximately 20,000 years ago with a more recent founder effect explaining the high incidence of the disease in Scandinavia.

Following the momentous discovery that TDP-43 was the dominant ubiquitinated protein within inclusions in ALS and non-tau FTD, Dr. Shaw’s team sequenced TARDBP in their cohort of ALS cases and was the first to identify mutations in familial and sporadic ALS. He demonstrated that the mutations were associated with increased TDP-43 cleavage in transfected neurons and were neurotoxic in the embryonic chick spinal cord. His team has focused on understanding how these mutations lead to the disease and, in collaboration with others, is characterizing cellular zebrafish, fruit-fly and mouse TDP-43 models. Using genome-wide linkage, he identified a novel locus for familial ALS on Chromosome 16q.

In collaboration with Dr. Robert Brown, his team identified three different mutations in Fused in Sarcoma (FUS) in eight dominant ALS kindreds accounting for approximately 3% of all familial cases. They were the first group to demonstrate linkage to Chromosome 9p13-21 in a large Dutch kindred with ALS and FTD. Ten kindreds were subsequently linked to this locus where recently a massive hexanucleotide repeat expansion mutation was identified in C9ORF72 by Drs. Rosa Rademaker and Bryan Traynor. In the King’s College ALS cohort, expansions account for approximately 20% of familial and 5% of sporadic ALS. Current evidence implicates RNA-mediated toxicity, which with TDP-43 and FUS, places the disruption RNA processing center-stage in the pathogenesis of ALS and FTD.

"I am delighted that Professor Shaw is this year’s Sheila Essey Award recipient,” said ALS Association Chief Scientist Lucie Bruijn, Ph.D. “His outstanding contributions to the genetics of ALS mark a significant time in ALS research and increased potential to find meaningful new treatments for the disease."

The $25,000 prize honors the memory of Sheila Essey and was made possible through the generosity of the Essex Family Fund. Past recipients have often used the funds to support research of promising young scientists on their teams. To see a list of these recipients, visit http://www.alsa.org/research/researchers/sheila-essey-award/.

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**RESOURCES**

- ALS mutations database [http://alsod.sp.kcl.ac.uk/index.aspx](http://alsod.sp.kcl.ac.uk/index.aspx)
- Coriell NINDS DNA repository [http://ccr.coriell.org/nindis/](http://ccr.coriell.org/nindis/)
- ALS Epidemiology [http://aces.stanford.edu/FrElRe.html](http://aces.stanford.edu/FrElRe.html)
- SOD1 mutant mice, Jackson Laboratory mouse models [http://farmice.jax.org/index.html](http://farmice.jax.org/index.html)
- Control and SOD1 fibroblasts [http://ccr.coriell.org/Sections/Search/Advanced_search.cfm?P08d175&c=Coriell&k=SU](http://ccr.coriell.org/Sections/Search/Advanced_search.cfm?P08d175&c=Coriell&k=SU)
Robotic Screening of Motor Neurons

One hurdle in drug development is the need to examine individual cells day after day to determine a therapeutic effect of target compounds. An approach to make that process more efficient was described by Steve Finkbeiner, M.D., Ph.D., of the Gladstone Institute in San Francisco. The system uses a robotic arm to carry cell plates from the incubator to the microscope, allowing precise repeatability and fully automated data acquisition. “We can observe individual cells over many hours,” he said. “We can also harness well-to-well variability to extract more information about cells.” The system is ideal for working with induced pluripotent stem cells, generated from individual ALS patients. He is currently screening for compounds that affect autophagy, the lysosome-based recycling pathway that may be affected in ALS.

Robotic screening of motor neurons
—Image courtesy of Chris Goodfellow

Drug Discovery Workshop

Continued from page 1

clinical trials. “In between lies the zone of chaos,” Dr. Austin said ruefully, where the costs of proceeding skyrocket and the likelihood of success plummets. “We don’t understand what the rules are here.”

The ALS Association and similar non-profits play an essential role in bridging that gap, he said, since they can help fund risky development research directly, and perhaps just as important, facilitate collaborations among basic researchers, biotech companies, and “Big Pharma” to move promising drugs forward.

The NIH has multiple programs designed to promote the same goals, including the National Institutes of Health, Center for Translational Therapeutics, which provides expertise in translational drug development, including chemical genomics, quantitative high-throughput screening and preclinical development. “We try to get projects to the point of low-enough risk that Pharma will accept the risk and begin to develop the project,” Dr. Austin said. More information is available at http://nctt.nih.gov.

The landscape for drug development has changed among the big pharmaceutical companies, said Stephen Freedman, Ph.D., Director of the Gladstone Center for Translational Research at the Gladstone Institute, an independent, non-profit biomedical research institute in San Francisco. “Mergers have created large companies that are slow and inflexible. They are increasingly looking outward to small biotechs to supplement their pipelines,” said Dr. Freedman. Those companies, in turn, often depend on academic researchers to drive new developments. Freedman added that many who work in academia and some in biotechs lack the understanding of what makes a program valuable to Pharma. These individuals often “oversell” the scientific rationale and pay little attention to the practical aspects of bringing a new agent to trials. Challenges often involve safety profiles, the use of pharmacokinetics, and intellectual property claims.

The Crucial Need for Biomarkers

A key aspect of a successful development program, many speakers agreed, is the availability of validated biomarkers that can be used in a clinical trial to supplement functional endpoints, such as

Continued on page 5
Drug Discovery Workshop  
Continued from page 4

Robert Bowser, Ph.D., of the Barrow Neurological Institute in Phoenix, Ariz., is hunting for proteins in the cerebrospinal fluid (CSF) of ALS patients that may signal the onset of disease. He combines liquid chromatography and mass spectrometry to separate and identify CSF proteins, controlling for patient age, gender, site of onset, and other variables. “There are more than four thousand proteins in the human CSF,” he said, meaning he is hunting for a small signal among a very noisy background, and he may have found one.

By comparing patients to controls, he has found that the ratio of the levels of phosphorylated neurofilament heavy chain to complement C3 (pNFH/C3) identifies ALS patients with a sensitivity of 96% and a specificity of 90%. At the moment, the ratio is diagnostic, not prognostic, he said, since finding a marker of disease progression would require longitudinal samples from individual patients. That work is ongoing with some preliminary evidence that the protease inhibitor cystatin C may help distinguish slow from fast progressors.

Rita Sattler, Ph.D., of Johns Hopkins University in Baltimore, Md., John Gerdes Ph.D. and Richard Bridges, Ph.D., of the University of Montana, and Henry Van Broeckin, Ph.D., of the University of California San Francisco have collaborated on the discovery and development of a PET ligand that tags the EAAT2 glutamate transporter, which they are developing as a potential therapeutic biomarker for treatments that increase the level of the transporter. Glutamate is an excitatory neurotransmitter, and severe loss of EAAT2 on astrocytes is seen in ALS patients, leading to the hypothesis that it may hasten motor neuron death. In animal models, transporter loss precedes symptom onset. Restoring the transporter has the potential to be therapeutic, a strategy currently being explored in a Phase III clinical trial of ceftriaxone.

The search for a PET ligand for EAAT2 began in 2006, Dr. Gerdes said. The agent this team has developed is a glutamate transport inhibitor, given as a pro-drug which is hydrolyzed to the active ligand binding form after it crosses the blood-brain barrier. It is tagged with radioactive fluorine and has a half-life of 110 minutes, allowing a cyclotron-to-patient window of 3.5 hours. Work in the rat is almost completed, with testing in monkeys next. They plan to do human safety testing later in 2012, and, if all goes well, hope to use it as a biomarker as early as 2013. “We are on the home stretch,” Dr. Gerdes said. “Developing a PET imaging agent takes at least five years. I think we will have our answer this year.”

Restoring the transporter has the potential to be therapeutic, a strategy currently being explored in a Phase III clinical trial of ceftriaxone.

New Insights into ALS Disease Mechanisms

“The last eight months have been the most exciting in the history of ALS genetics,” said Don Cleveland, Ph.D., of the University of California at San Diego. The discovery of the C9ORF72 gene, responsible for a large majority of familial ALS and a smaller proportion of sporadic cases, has galvanized the field to look even more deeply at the role of RNA in the disease. At the same time, the X-linked ubiquilin-2 gene, while responsible for fewer cases, has again highlighted the likely importance of protein handling in pathogenesis.

The discovery of the expanded hexanucleotide repeat in the C9ORF72 gene, and the suggestion that it forms large RNA inclu- sions, has led to the question of whether this form of ALS might be similar to myotonic dystrophy, in which toxic RNA foci bind a transcription factor, leading to dysregulation in multiple cell types. However, a note of caution was offered by one of the co-discoverers of the gene. “The original data on inclusions has so far been difficult to replicate,” continued Bryan Traynor, M.D., Ph.D., of the National Institutes of Health. “We don’t yet know the relevance..."

Continued on page 6

SOD1 gene mutation (chromosome 21) discovered in familial ALS
Trials using glutamate blocker riluzole begin
Animal studies combining CNTF and BDNF demonstrate decreased motor neuron loss
GDNF rescues degenerating motor neurons during development in an in vitro experiment
Toxic properties of the SOD1 enzyme discovered and linked to familial ALS
FDA approves riluzole
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 for compound screening
The ALS Association approves funding for ALS-specific research

Zebrafish with insert in axonal branching. Zebrafish provide a useful screening tool to identify novel targets and for compound screening.

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Drug Screening in Zebrafish

Wim Robberecht, M.D., Ph.D., has developed a drug screening system using zebrafish. These freshwater vertebrates develop in a few days, grow to only an inch long, and are transparent, allowing the growth of their motor neurons to be observed in vivo. Dr. Robberecht has found antisense molecules that rescue fish mutant for TDP-43, an RNA-binding protein that causes ALS and that is found in neuronal aggregates. The most effective one targets a receptor in the ephrin system, a developmental guidance system for growing motor neurons. “Knocking down the receptor rescues the phenotype,” he said, although the interactions between TDP-43 and ephrin are still unclear. Deletion of one copy of the receptor in SOD1 mice increased survival by 50%. A similar beneficial effect in fish can be induced with a small molecule that targets the receptor. The potential importance of the receptor as a target in ALS is also highlighted, Dr. Robberecht said, by the discovery of an inverse correlation between the expression of the receptor and survival of ALS patients.

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

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
REQUEST FOR PROPOSALS

Clinical Research Pilot Study

Deadline for brief study outline: May 28, 2012
Email researchgrants@alsa-national.org for study outline form.

- The ALS Association’s scientific research program TREAT ALS (Translational Research Advancing Therapy for ALS) encourages translational research to bring new treatments to patients.
- Currently there is only one FDA approved drug for ALS, riluzole, which has a modest effect on patient survival.
- This request for proposals (RFP) seeks to fund pilot clinical studies to obtain preliminary clinical data which will support applications to the National Institutes of Neurological Disorders and Stroke (NINDS) for subsequent larger clinical trials of an intervention to treat or prevent ALS.
- The proposed study must address questions that, when answered, will optimize the design of more definitive clinical trials rather than simply addressing the clinical question with lower power. The research proposal should directly address how the preliminary study will advance the design of a subsequent definitive clinical trial for efficacy.

Budget: A maximum of $150,000/year for a maximum of two years
No indirect costs will be paid for these awards

Application Receipt Dates:
- Brief study outline: May 28, 2012
- Request to submit full application: June 29, 2012
- Submission of full application: September 10, 2012
- Notification of award: November 30, 2012

Funding begins upon receipt of relevant signatures.

Drug Discovery Workshop

Continued from page 5

of RNA inclusions.” A loss of function of the gene is also possible; the normal function of the gene is unknown.

Meanwhile, Dr. Cleveland brought attention to a recent result reinforcing the idea that astrocytes are toxic in ALS, and that the effect may be mediated through SOD1, even in patients with the sporadic form of the disease. “It is very important to replicate this. If it is true that normal SOD1 drives toxicity, then antisense oligonucleotides become broadly applicable in sporadic disease.” Such oligonucleotides are currently in clinical trials in familial disease (see sidebar on page 1).

SOD1 aggregates, and how to prevent them, are a focus of three groups who presented their results at the meeting. “For the vast majority of the proteome, folding is biologically assisted,” said Jeffrey Kelly, Ph.D., of the Scripps Research Institute in La Jolla, California. Chaperones help with folding, while proteasomes and the autophagy pathway dispose of misfolded or aggregated proteins. Dr. Kelly’s group looks for small-molecule activators of protein...antibodies to misfolded SOD1 may have the potential to reduce levels of toxic protein and slow disease progression.

Several preclinical studies have been performed, he said, “but so far there is no evidence of changes in misfolded protein in most studies. This is disappointing, although it may mean that the models are too aggressive,” with more SOD1 than the antibodies can handle. “Is this a validated target for familial ALS? Probably so, but we will need more confirmation for sporadic ALS.”

Continued on page 7

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 dysfunction may play an important role in ALS
- Study funded by The ALS Association to find biomarkers in cerebrospinal fluid and blood
- Ceftriaxone increases levels of the glutamate transporter GLT1 in a mouse model of ALS
- First international workshop on frontotemporal dementia discussions 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
- 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

2005
- Stem cell study shows SOD1 mutant support cells can kill any motor neuron
- AL S 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

2006

2007
Jean-Pierre Julien, Ph.D., reported that in his work with AAV delivery of single-chain recombinant antibodies against misfolded SOD1, delivered intrathecally into SOD1 mice, antibody can be detected within three weeks of treatment. Treatment increases lifespan by about 16 days, although with high variability. Future work will be needed to confirm the mechanism of the benefit, he said. In addition, he presented evidence that TDP-43 deregulation in ALS can contribute to pathogenesis through activation of nuclear factor-kB (NF-kB) p65, a key molecule of the innate immune response. Inhibition of this pathway by Withaferin A treatment conferred protective effects in TDP-43 transgenic mouse models of ALS.

Meanwhile, Pamela Shaw, M.D., of the University of Sheffield, UK, is investigating a stress-response pathway, called Nrf2-ARE, which drives expression of a large group of protective genes, and is downregulated in SOD1 mice. “We thought we might be able to protect by upregulating this system,” she said, “so we set out to identify small molecules that had this effect.” A drug screen led her to S(+)-apomorphine, the chemical cousin of an antiparkinson drug, but without dopaminergic activity. In mice, treatment led to better motor performance even late in the disease, but, for unknown reasons, no improvement in survival.

“We are in a very exciting time for understanding ALS. By bringing together so many people dedicated to discovering new treatments for the disease, we think we can maximize the chances of moving promising therapies to the clinic for testing in patients.”

—Lucie Bruijn, Ph.D.

“Activation of this pathway is an attractive target,” Dr. Shaw said. Future work will include looking more closely at the downstream events the drug causes, and trying to find the reasons for its differential effects on function and survival.

“We are in a very exciting time for understanding ALS,” said ALS Association Chief Scientist Lucie Bruijn, Ph.D., who organized the meeting. “By bringing together so many people dedicated to discovering new treatments for the disease, we think we can maximize the chances of moving promising therapies to the clinic for testing in patients. Everyone recognizes this is a hard problem, but the interest and dedication in the field to solving it is intense, and the willingness of these different groups to work together on it increases the odds we will make progress.”

AAN Foundation—ALS Association
Clinician-Scientist Development Three-Year Award
Co-sponsored by the American Academy of Neurology Foundation and The ALS Association
Application Deadline: October 1, 2012
The American Academy of Neurology (AAN) Foundation and The ALS Association are pleased to announce a three-year Richard Olney, M.D., Clinician-Scientist Development Award to support a clinician-scientist’s research related to amyotrophic lateral sclerosis. The award aims to recognize the importance of good clinical research and encourage young investigators in clinical studies.

The three-year award will consist of an annual salary of $75,000, plus $5,000 in educational expenses, per year. Only direct costs will be funded by this award.

Eligibility
1. Must be a neurologist interested in an academic career in clinical research. Applicants must hold an M.D., D.O., or equivalent clinical degree from an accredited institution. Must be an AAN member of the AAN. Disease related studies not directly involving humans or human tissue also are encouraged if the primary goal is the development of therapies, diagnostic tests, or other tools to prevent or mitigate neurological diseases.
2. Applicants must have completed residency training but be less than five years from completion of residency when funding begins.

Evaluation and Selection
Applications are evaluated by members of the Clinical Research Subcommittee, Translational Neuroscience Subcommittee, and various ad-hoc reviewers based on the following criteria:
• Applicant’s ability ad promise as a clinician-scientist based on previous training and career plan, letters of reference, and curriculum vitae (30 percent)
• Quality and nature of the training to be provided and the institutional, departmental, and mentor-specific training environment (30 percent)
• Quality and originality of the research plan (40 percent)

Evaluations will be forwarded to the AAN Foundation Research Council for final determination of the award. Funding begins within 30 days after executing a signed agreement with the American Academy of Neurology Foundation and the recipient’s institution.

All materials must be submitted online. Apply now at www.aan.com/view/CRTF. Notification of recipients: January 2013.
Role of FUS in ALS

Fused in Sarcoma (FUS) is an RNA-binding protein involved in many processes of RNA metabolism. Mutations linked to about 4% of familial ALS cases have recently been identified. How these mutations in FUS lead to ALS remains unclear. Haining Zhu, Ph.D., Jianhang Jia, Ph.D., and colleagues demonstrated that over-expression of normal FUS protein and FUS protein carrying ALS-linked mutations led to motor degeneration and damage at the neuromuscular junction in a fly model. Production of normal and mutant FUS induced progressive toxicity in multiple tissues in a dose and age-dependent manner. Motor neurons degenerated through a cell death pathway called apoptosis. The investigators demonstrated that nuclear localization of the mutant protein was required for the induced toxicity.


Motor Neurons in Culture Generated from Induced Pluripotent Stem Cells Show a Phenotype

An international research team, led by the Euan MacDonald Centre for Motor Neuron Disease Research at the University of Edinburgh in partnership with researchers from King’s College London; Columbia University, New York; and the University of San Francisco, is the first to publish a phenotype in motor neurons derived from fibroblasts from a patient carrying a TDP-43 mutation. Abnormalities of a protein called TDP-43 are implicated in more than 90% of cases of ALS/MND. Recent technologies enabling the generation of motor neurons from induced pluripotent stem cells (iPSCs) has enabled investigators to develop human cell systems in culture for testing compounds to identify new therapies for ALS. iPSCs that carry the TDP-43 M357V mutation can be differentiated into functional motor neurons. Mutant neurons had elevated levels of soluble and detergent-resistant TDP-43 protein, decreased survival in longitudinal studies, and increased vulnerability to antagonism of the PI3K pathway. The investigators concluded that expression of physiological levels of TDP-43 in human neurons is sufficient to reveal a mutation-specific cell-autonomous phenotype providing a valuable tool for screening compounds to identify therapies for ALS.


Over-oxidized Form of Superoxide Dismutase in Sporadic Bulbar ALS Shares Toxic Mechanism with Mutant SOD1

A recent study explored the role of Cu/Zn Superoxide Dismutase 1 (SOD1) found in sporadic ALS with bulbar onset. The team led by ALS Association-funded investigator Piera Pasinelli, Ph.D., Thomas Jefferson University, Pennsylvania, asked whether post-translational modifications to SOD1 could lead to disease in sporadic ALS cases. Analysis of SOD1 isolated from patient-derived lymphoblasts–immature cells which typically change to form mature lymphocytes (white blood cells)—from a subset of bulbar onset patients by a technique called “immunoprecipitation” identified iper-oxidized SOD1, which is above baseline oxidation levels. Interestingly, this was seen in seven cases of bulbar ALS and not seen in four cases of familial ALS or 13 cases of lower and upper limb onset. Ten healthy controls were also included in the analysis. Similar to mutant SOD1-like properties, iper-oxidized SOD1 isolated from sporadic bulbar onset cases formed a toxic complex with mitochondrial Bcl 2, when further stressed. There was no correlation between the appearance of the iper-oxidized SOD1 and patient’s sex, age, or duration or progression of disease. However, it did correlate with bulbar onset, highlighting that the underlying mechanism in various subsets of ALS may differ and, with further validation of levels of iper-oxidation, may provide a biomarker to define various subsets of ALS as well as have therapeutic implications.

http://www.pnas.org/content/early/2012/03/09/1115402109.long

Previous studies suggest that misfolded SOD1 may not only be a cause of familial cases of ALS linked to SOD1 mutations but also a cause of sporadic ALS cases. In an August 2011 report, investigators led by Brian K. Kaspar, Ph.D., Ohio State University School of Medicine, concluded that astrocytes isolated from post-mortem cases of ALS resulted in motor neuron damage; furthermore, the damage could be reversed by lowering SOD1 in these astrocytes.


C9ORF72, ALS and FTD

The recent discovery of a hexanucleotide repeat expansion in C9ORF72 has been followed by numerous publications describing the pathology of ALS and frontotemporal dementia (FTD) carrying these repeat mutations. In a review article by Christine Van Broeckhoven and her colleagues, they described recent advances that support the existence of an FTD-ALS spectrum, with a focus on the several new genes recently identified and implicated in both ALS and FTD.


A group led by Pamela Shaw, M.D. at the Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, UK, described clinical and pathological phenotypes associated pathogenic C9ORF72 mutations in a cohort of 563 cases from Northern England, including 63 with a family history of amyotrophic lateral sclerosis. The C9ORF72 intronic expansion was present in 43% of familial cases and 7% cases with sporadic ALS. The disease duration was significantly shorter in cases with C9ORF72-related amyotrophic lateral sclerosis compared with non-C9ORF72 ALS. C9ORF72 cases included both limb and bulbar onset disease and all cases showed combined upper and lower motor neuron degeneration.


Magdalini Polymenidou, Ph.D. and colleagues review the recent genetic discoveries for ALS with a particular focus on the common causes of these diseases which include mutations in the RNA/DNA-binding proteins, TDP-43 and FUS/TLS and most recently, hexanucleotide expansions in the C9ORF72 gene, discoveries that highlight the overlapping pathogenic mechanisms that trigger ALS and FTD. TDP-43 and FUS/TLS, both of which participate in several steps of RNA processing, are abnormally aggregated and mislocalized in ALS and FTD, while the expansion in the C9ORF72 pre-mRNA strongly suggests sequestration of one or more RNA-binding proteins in pathologic RNA foci.


A group led by Bryan Traynor, M.D. who originally identified the repeat hexanucleotide repeat expansion, together with Rosa Rademakers, Ph.D., screened 4448 patients diagnosed with ALS (El Escorial criteria) and 1425 patients with FTD (Lund-Manchester criteria) from 17 regions worldwide for the GGGGCC hexanucleotide expansion using a repeat-primer PCR assay and determined that a common Mendelian genetic lesion in C9ORF72 is implicated in many cases of sporadic and familial ALS and FTD. Testing for this pathogenic expansion should be considered in the management and genetic counseling of patients with these fatal neurodegenerative diseases.