The big FUS in ALS

By Lev Osherovich, Senior Writer

Separate papers published in *Science* by researchers in the U.S.¹ and the U.K.² identify a new genetic player in amyotrophic lateral sclerosis by showing that mutations in an RNA-binding protein called FUS cause a rare familial form of the neurodegenerative disorder.

The findings support the idea that motor neuron disease could arise through any of a number of protein mishaps, making it unlikely that a single therapy would work for every case. However, drug developers are likely to hold off on targeting FUS until its relevance to the more common form of the disease—sporadic ALS—can be established.

ALS leads to the degeneration of motor neurons—the long nerve fibers that connect the spinal cord to muscles and control voluntary motion. About 90% of cases are sporadic, without a clear genetic basis. The remaining 10% are hereditary, with 20% of such cases caused by mutations in *superoxide dismutase 1* (*SOD1*).

Researchers have been hunting for other disease-causing mutations, and the *Science* articles were the result of two research teams' gene mapping efforts that converged on fusion (involved in t(12;16) in malignant liposarcoma) (*FUS*; *TLS*).

The studies were spurred by previous research that hinted at an ALS susceptibility locus on chromosome 16 (refs. 3,4) and were performed by independent teams at **King's College London** and **Massachusetts General Hospital** (MGH). Last year, the same King's College team identified another familial ALS gene, *TAR DNA binding protein 43* (*TARDBP*; *TDP-43*), which encodes another RNA-binding protein.⁵

The Boston team found that in two families with chromosome 16-linked ALS, the *FUS* gene bore missense mutations. Meanwhile, the London team detected *FUS* mutations in all ALS-stricken members of a large British family.

Collectively, the two groups identified 16 different *FUS* mutations among a total of 278 familial ALS patients. They found no such mutations in sporadic ALS patients or healthy controls.

Because *TDP-43*- and *SOD1*-linked ALS involve mislocalization of these proteins within the cell, the researchers suspected that a mutant *FUS* could result in a similar problem. Indeed, in cell culture, fluorescently labeled versions of mutant *FUS* proteins localized primarily to the cytoplasm, whereas wild-type protein went into the nucleus.

Both teams also saw cytoplasmic mislocalization of *FUS* protein in brain tissue from *FUS*-linked familial ALS patients. According to Christopher Shaw, this is similar to what happens to *TDP-43* in familial

ALS cases linked to that gene.

Shaw is a professor of neurology at the Institute of Psychiatry at King's College and lead author of one of the two papers. The MGH team was led by Thomas Kwiatkowski, Jr., and Robert Brown, Jr. Kwiatkowski is an instructor of neurology at MGH and Brown is now a professor of neurology at the **University of Massachusetts Worcester**.

Many mechanisms

With the identification of *FUS*, the two papers solidify the hypothesis that problems with any number of RNA-binding proteins could lead to ALS. If this number continues to grow, ALS patients may in fact be a heterogeneous population requiring highly personalized therapeutic strategies. Alternatively, companies may want to focus less on fixing individual disease-related proteins and instead on sustaining sick neurons.

The findings “reflect the complexity of the pathology of the disease and suggest that multiple mechanisms may lead to ALS,” said Daniel Lee, a scientist at **GlaxoSmithKline plc**'s neurodegenerative disease research group. “These various disease gene mutations may result in some common phenotype, though the molecular mechanisms appear to be distinct.”

GlaxoSmithKline has a human genetics research program to characterize genetic variation that influences drug response.⁶

Shaw thinks that mishaps with *FUS*, *TDP-43* and potentially other RNA-binding proteins could cause a spectrum of motor neuron

diseases, including familial and sporadic forms of ALS and a related condition, spinomuscular atrophy (*SMA*), which is linked to another RNA-binding protein called survival of motor neuron 1 telomeric (*SMN1*; *SMN*). He noted that all of these proteins normally work together in the nucleus to aid RNA processing but appear to cause disease when forced out of the nucleus due to mutations.

Shaw said one hypothesis to explain both ALS and *SMA* is that mislocalization of nuclear players like *TDP-43* and *FUS* disrupts RNA metabolism and somehow poisons motor neurons.

Jeff Rothstein, professor of neurology at **The Johns Hopkins University**, thinks the findings published in *Science* help build the case for RNA processing defects as a cause of motor neuron diseases. In 1998, Rothstein's team linked certain features of ALS pathology to RNA processing problems.⁷

“People have tried to link ALS to *SMN1* pathology, but this never went anywhere,” he said. “Last year, Chris Shaw came out with the *TDP-43* story, which resurrected the RNA metabolism hypothesis. Here, Shaw and Brown have found another gene involved with RNA metabolism” and ALS.

Virginia Lee, professor of pathology and lab medicine at the **University of Pennsylvania School of Medicine**, also suspects that motor neuron diseases could arise through any of several RNA-related defects.

“These various disease gene mutations may result in some common phenotype, though the molecular mechanisms appear to be distinct.”
—Daniel Lee, GlaxoSmithKline plc

“For 15 years we have had only one gene to work with—SOD1—but now we have multiple genes,” she said. “The idea that many RNA-binding proteins could cause the clinical phenotypes of ALS is a reasonable hypothesis.”

This hypothesis could be validated by the identification of other familial ALS genes. Virginia Lee said previous gene-mapping studies have pointed to as yet uncloned genes on chromosomes 9 and 18 that underlie other cases of familial ALS and frontotemporal dementia, another neurodegenerative disorder that is also linked to TDP-43.

“If these genes also turn out to be RNA-binding proteins, this would be very interesting,” she added.

Keeping it familial

The challenge now is to figure out the physiological consequences of FUS mislocalization in ALS. It is still unclear whether the neurodegenerative effects of mutant FUS result from the loss of the protein's normal function or from a dominant negative effect on other proteins besides TDP-43, whose localization appears to be unaffected by FUS mutations.

Distinguishing between the gain- and loss-of-function effects of FUS mutations will require mouse studies. Although knockouts of FUS have been made, “they're either lethal or are very sick,” said Brown.

Brown plans to engineer conditional knockouts of FUS and knock-ins of the mutant *FUS* gene and will look for ALS-like phenotypes among them.

The biggest question, however, is whether FUS plays a role in sporadic ALS.

“Sporadic ALS has been such an enigma. It's been so hard to get a foothold on the genetic and environmental determinants” of sporadic ALS because of slow progress with existing mouse models of SOD1-linked disease, Kwiatkowski told *SciBX*. “It's premature to say whether FUS could be a target for the majority of ALS cases.”

Nonetheless, Brown suspects that FUS could be involved in sporadic ALS and plans to examine the localization and function of the protein in nerve tissue from sporadic ALS patients.

Kwiatkowski plans to characterize changes in axonal RNA transport and mitochondrial energy metabolism caused by FUS mutations. He said preliminary evidence suggests that FUS-related problems with these processes could tie it in to SOD1.

Meanwhile, Shaw's team is now studying the role of FUS and other RNA-binding proteins in frontotemporal dementia.

Shaw did not patent his team's finding. Brown has filed a patent application for his group's discoveries and said it is available for licensing through MGH.

Going long

For companies in the ALS space, the discovery of FUS is unlikely to

prompt a quick change of course away from the current strategies of targeting SOD1 or attempting to boost neuron survival.

However, if there are indeed multiple unrelated ways to destabilize the health of motor neurons, this could bolster the case for increasing neuronal survival, said Philip Gregory, VP of research at **Sangamo BioSciences Inc.**

“ALS presents in motor neurons, which are some of the longest cells in the body. There may be any of a number of ways to disrupt the health of these cells,” he said. “Generalized protection against nerve death may be useful against this slow-developing type of insult. This suggests that we should be trying protective factors such as our therapeutic, which attempts to make more VEGFA.”

Sangamo's SB-509 is a zinc-finger protein therapeutic that boosts the transcription of VEGFA, which in turn activates α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) and promotes nerve cell proliferation and regeneration. SB-509 is in Phase II testing to treat ALS.

Gregory noted that the company's ALS trial protocol doesn't distinguish between familial and sporadic cases because the underlying cause of the disorder may not matter for the therapy to work.

“We think that by providing a factor with multiple modes of action including both angiogenic and direct neuroprotective effects, we will protect the cells from whatever insult they are challenged by,” he said.

Osherovich, L. *SciBX* 2(11); doi:10.1038/scibx.2009.432
Published online March 19, 2009

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