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## Genetic Stutter Increases Risk of ALS

The causes of the devastating neurodegenerative disease amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) are largely mysterious. Five to ten percent of cases are inherited, but most people who develop ALS have no relatives with it. A new study from Howard Hughes Medical Institute (HHMI) scientists and colleagues, however, has found a genetic risk factor that appears to be associated with about five percent of cases.

The distorted gene identified in the study is among the most common genetic risk factors so far defined for the disease, which afflicts about 30,000 people in the United States. Perhaps more importantly, the discovery offers insight into the usual progression of ALS, suggesting avenues for further research and perhaps treatments.

Like most diseases, ALS starts out small: Curious clumps of proteins accumulate in the motor neurons of the spinal column, junk marked for disposal that for some reason the cells fail to degrade. The arms or legs grow weak; muscles twitch. Swallowing becomes difficult, and finally, the limbs and then the body stop obeying brain signals until even breathing requires mechanical assistance. The only treatment, a drug called riluzole, slows disease progression moderately. There is no cure.

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- Nancy M. Bonini

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Using humble laboratory aids -- yeast and fruit flies -- HHMI investigator Nancy Bonini, a professor of biology at the University of Pennsylvania, and collaborators have unlocked one secret about the protein clumps associated with the fast-moving degenerative disease. UPenn assistant professor Aaron D. Gitler, who uses yeast models to study neurodegenerative diseases, is a co-senior author on the paper. Other collaborators are from the Center for

Neurodegenerative Disease Research at UPenn, and from Goethe University in Frankfurt, Germany, published its findings in the August 26, 2010, issue of the journal *Nature*.

The guilty gene Bonini and colleagues uncovered is called Ataxin-2, already known to play a role in another neurodegenerative disease called spinocerebellar ataxia type 2 (SCA2).

“What I find really exciting about this is that a significant risk factor for a human disease came from very simple genetics,” Bonini said. “That really highlights the exceptional power of these systems, and how they can be used to provide novel insight into human disease.”

The protein-building information that genes carry is encoded in the specific sequence of four chemicals called nucleotides, represented by the letters A for adenine, T for thymine, G for guanine, and C for cytosine. The arrangement of these letters dictates the recipe for the amino acids that together form a protein. In patients with SCA2, the Ataxin-2 gene stutters on a sequence of three letters: CAG, which together tell the cell to make the amino acid glutamine. In patients with the disease, this sequence can repeat more than 34 times, misdirecting the cell to manufacture Ataxin-2 with too much glutamine.

A scan of DNA from 980 people with neither ALS nor SCA revealed that in most individuals, the Ataxin-2 gene has 22 or 23 CAG repeats. But the team found that in almost five percent of samples from more than 900 patients with ALS, the gene carried between 27 and 33 CAG repeats. So the stutter in Ataxin-2 associated with ALS patients is longer than typically found, although shorter than what causes the human disease SCA2.

With Ataxin-2 as a starting point, “you can then extrapolate more broadly,” Bonini said. For instance, she explained, the findings suggest that even normal Ataxin-2 may contribute to the clumping associated with ALS when it combines with cell changes brought on by age and physiological stressors.

The work began when Gitler’s lab used yeast to screen for genes that affected the toxicity of a protein called TDP-43, which UPenn researchers had discovered accumulates abnormally in the cell cytoplasm in ALS patients. Cytoplasm is the fluid surrounding a cell’s nucleus, where most of the cell’s chemical processes take place.

Of the 5,100 genes Gitler’s group tested, 27 increased the toxicity of TDP-43, including the yeast version of Ataxin-2. Bonini said the finding was “tantalizing.”

“I had already worked with Ataxin-2 in other studies. We knew it was a human disease gene.” The next step was to see what it would do in the nervous system of fruit flies. Fruit flies are ideal experimental organisms,

because they have complex brains and behaviors and most of the gene pathways found in flies are also found in humans.

Their fly experiments revealed that higher levels of Ataxin-2 were associated with more severe neurodegeneration, as well as a shorter lifespan. Conversely, reducing Ataxin-2 levels reduced the level of disease.

The researchers discovered that Ataxin-2 formed a complex with TDP-43 using RNA as a bridge between the two proteins. They further tested Ataxin-2 and TDP-43 in human cells and saw evidence for similar interactions as in yeast and fruit flies.

Intriguingly, it appears that Ataxin-2 need not have an abnormal number of CAG repeats to make trouble. “Based on the fly data, I suspect there’s a bad interaction between TDP-43 and the normal Ataxin-2,” Bonini said. “You don’t actually need the repeat expansion to interact in a deleterious manner. But when you have the expanded repeat, the interaction is worse. That’s what we suspect.”

To test this idea, the researchers in Gitler’s group put cells under heat stress, increasing the cell temperature transiently. Under this physiological stress, TDP-43, which is normally in the nucleus, moves in part to the cytoplasm; it moved more in cells with Ataxin-2 stutters. “I believe what the data are trying to tell us here is that the interaction that’s going on between these two proteins is worse if you have a longer-length Ataxin-2 [CAG] repeat,” Bonini said.

For Bonini, the discoveries are an important step forward, but also continued proof of concept. “I truly believe that simple experimental systems like yeast and the fly are making a difference to our understanding of ALS and other diseases, and will provide the foundation for new treatments,” Bonini said. “I think it’s pretty incredible. To me it says, ‘never underestimate the power of yeast and fly.’”