Medical Xpress

Genetic screening in yeast reveals new candidate gene for Lou Gehrig's disease

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Two RNA-binding proteins, TDP-43 and FUS/TLS, are associated with the human motor neuron disease amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease). Julien Couthouis et al. present evidence that these two proteins might represent the "tip of the iceberg" and that additional RNA-binding proteins with similar properties could also contribute to the disease. Credit: Image courtesy of Stanley H. Gitler, CPA.

(Medical Xpress) -- Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is a universally fatal neurodegenerative disease. Mutations in two related proteins, TDP-43 and FUS, cause some forms of ALS. Specifically, these two proteins are RNA-binding proteins that connect to RNA to regulate the translation of proteins and other cellular functions such as RNA splicing and editing. In a new study, researchers at the Perelman School of Medicine at the University of Pennsylvania discovered additional human genes with properties similar to TDP-43

and FUS that might also contribute to ALS.

There are over 200 human RNA-binding proteins, including FUS and TDP-43, raising the possibility that additional RNA-binding proteins might contribute to ALS pathology. Using yeast as a model organism, the lab of cell biologist and senior author Aaron Gitler, PhD, assistant professor of Cell and Developmental Biology, surveyed 133 of these proteins. They further winnowed the candidates using bioinformatics to about 10 proteins, selecting one called TAF15, to study further. Their findings appear this week in the Proceedings of the National Academy of Sciences.

A group of high school students performed the screening in the yeast cells in the Gitler lab during the summer of 2009 with the aim to more rapidly locate disease genes associated with ALS. They were from Philadelphia area schools - J.R. Masterman Laboratory and Preparation School, Central Bucks High School East, Radnor High School, and Conestoga High School - and most of the students have gone on to pre-medical programs or biology majors in college.

"We used yeast to predict new ALS disease genes," explains Gitler. "TDP-43 and FUS are both RNA-binding proteins involved in ALS and we have found previously that they both aggregate and are toxic in yeast. We wondered if there were more proteins like these out there and, if so, we probably could find them using our yeast screening approach. We performed a large screen for other human RNA-binding proteins with properties similar to TDP-43 and FUS in yeast and then validated one by sequencing the DNA of ALS patients. We also did experiments in cells and fruit flies to see how the new protein functioned."

Collaboration was Key

From DNA derived from 735 sporadic ALS patients and over 1300

controls, the team found mutations in TAF15 that were absent in healthy controls. These variants, when expressed in cultures of rat embryo spinal cord neurons, caused the protein to clump in the cytoplasm of the neurons, similar to clumping seen with TDP-43 and FUS. What's more, working with Nancy Bonini, PhD, a Penn professor of Biology, they found that TAF15 initiated neurodegeneration in fruit flies. Bonini is also an Investigator of the Howard Hughes Medical Institute.

The lab of James Shorter, PhD, assistant professor of Biochemistry and Biophysics, provided more clues about TAF15. In an aggregation assay using purified TAF15 protein, they found that mutant versions of TAF15 clumped faster. The purified TAF15 protein also harbored a prion-like portion in its amino acid sequence, much like TDP-43 and FUS, which might be a future predictor of neurodegenerative qualities in mutant proteins.

The lab of Zissimos Mourelatos, MD, associate professor of Pathology and Laboratory Medicine, tested effects of TAF15 in cell cultures of neurons derived from mouse embryonic stem cells and found that TAF15 mutant versions accumulated abnormally in the cytoplasm of these cells.

Finally, looking at post mortem samples from sporadic ALS patients in the tissue bank maintained by co-authors John Trojanowski, MD, PhD, director of the Penn Institute on Aging, and Virginia Lee, PhD, director of the Center for Neurodegenerative Disease Research at Penn, the team found clumps of TAF15 accumulated in human spinal cord neurons in these ALS patients.

Taking all of these lines of evidence into account, the authors propose that aggregation of RNA-binding proteins might contribute very broadly to ALS pathology. Looking forward, Gitler hopes that, “the genes identified in the yeast screen, coupled with prion-like domain prediction

analysis, will provide a powerful resource to speed ALS disease gene discovery."

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