Key gene behind hallmark of Lou Gehrig's disease identified

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Inside the brains of patients with amyotrophic lateral sclerosis, a debilitating neurodegenerative disease, is a telltale sign that marks almost every case: clumps of toxic proteins.

Now, researchers from the Stanford University School of Medicine and their collaborators have pinpointed a key gene behind the formation of one type of these neuron-damaging aggregates. They've also shown how

inhibiting the gene's function curbs production of the harmful protein.

"We know that these protein-rich aggregates are a clear hallmark of ALS," said Aaron Gitler, Ph.D., professor of genetics. "But this finding allows us a deeper look into how those aggregates are made, and potentially how we can hinder that process."

The gene, RPS25, codes for a piece of cellular machinery necessary for creating the protein-based gunk that amasses in some forms of ALS and damages healthy neurons. When the gene's activity was experimentally depleted—in yeast, in neurons derived from patients with ALS and in fruit flies—Gitler and his team saw levels of the lethal protein drop by about 50 percent across the board.

The team also tested the function of RPS25 in human cells modeling Huntington's disease and spinocerebellar ataxia, two other neurodegenerative illnesses that have protein-aggregate "hallmarks" similar to ALS, said Shizuka Yamada, a graduate student in Gitler's lab. There, too, inhibiting the gene helped tamp down the levels of bad protein.

It's still early days, Yamada said, but hampering the RPS25 gene seems like a promising target for reducing the destructive proteins seen in ALS and even extending life span, as was seen in the fruit fly model of ALS with low activity levels of the gene.

A paper detailing the results of the research will be published July 29 in *Nature Neuroscience*. Gitler, who holds the Stanford Medicine Basic Science Professorship, is the senior author. Yamada is the lead author.

An alternate route

Also known as Lou Gehrig's disease, ALS is a condition that kills off

motor neurons, which are crucial to all physical tasks, from brushing one's hair to breathing. The root cause behind every case is not always the same; there's a slew of genetic factors that play into the onset of ALS. Yet one gene is often the culprit. In ALS, it harbors a string of DNA that erroneously repeats itself.

It's these DNA repeats that are transformed into the harmful proteins that build up in the brain. As the proteins amass, they interfere with healthy neurons, blocking the cells' ability to function normally.

Outside of their toxic properties, what's notable about the protein aggregates is that they aren't made like other proteins found in the body, Yamada said. "These repeats actually shouldn't be made into proteins at all," she said. "They come from DNA that isn't supposed to code for anything, and yet somehow the proteins come to be anyway."

During run-of-the-mill protein formation, the ribosome, a sort of molecular machine that resides in the cell, processes messenger RNA, which contains genetic code based on DNA, and turns it into the raw materials of a protein. That process is called translation, and it's initiated by a code in the mRNA that shows the ribosome where to start translating. The ALS-associated DNA repeats don't have that start code, unlike normal mRNA.

"So regular translation doesn't work with the repeats," Yamada said. But it turns out there's a molecular workaround: an unconventional translation process called repeat-associated non-AUG translation, or RAN translation, that turns the ALS repeats into destructive protein bodies.

Putting the brakes on RPS25

The exact mechanism of RAN translation and its role in human biology

is not clear, but scientists do know that it still depends on the ribosome. To better understand the process, Gitler and Yamada turned to yeast, a simple organism that still has the major proteins and pathways of human cells. One at a time, the researchers decreased the function of individual yeast genes and monitored the fungus' RAN function. When subdued, several genes swayed RAN function, but one in particular, RPS25, stood out. With the gene hindered, production of the toxic protein fell by 50 percent.

The researchers also saw a 50 percent dip in the toxic protein when they tested how neurons derived from patients with ALS fared without RPS25.

"We were really excited to see the decrease in repeat proteins carry over into human cells," Yamada said. "It's always pretty cool when yeast biology can directly inform human biology." Because these cells came from patients who suffer from ALS, the research offered a reliable glimpse into how the neurons of individuals with ALS would respond to lower levels of RPS25, she said.

"Through genomic analyses, we could see that the ALS-associated repeats were still there; the sequences hadn't changed," Yamada said. "What was changing was the output of the ribosome; the repeats weren't being made into toxic proteins nearly as often."

Slashing a part of the cell's protein-making machine might sound risky, but it turns out a defunct RPS25 gene doesn't spoil normal protein production. Yet the researchers also showed that an inactive RPS25 gene affects more than just the ALS repeats; the dysfunctional gene similarly stunted erroneous protein production in cellular models of Huntington's disease and spinocerebellar ataxia, two neurodegenerative illnesses that have hallmark protein aggregates similar to ALS.

Moving toward more complexity

Finally, the researchers turned to fruit fly models of ALS to investigate how depleting RPS25 affected the insect overall. Not only did they see a similar decrease in toxic protein levels, they also saw an increased life span in the flies that lacked fully functional RPS25. Flies that harbored both the ALS mutation and a working RPS25 gene died by day 29, on average, while those that had the ALS mutation and lower amounts of RPS25 lived on average for 38 days. A healthy fruit fly lives about 50 days on average.

The findings are intriguing, Yamada said, but before the scientists can begin to pursue RPS25 as a drug target, the team has a couple boxes to tick off. The team now is investigating how a more complex animal model—like a mouse—would fair without RPS25.

"With the fruit flies, we tampered with the gene; we didn't remove it completely," Yamada said. "Whether an animal can survive without the gene entirely is a big part of our next step."

Furthermore, Yamada said, she and Gitler are still after a clearer picture of RAN translation in humans, overall. "Does it only occur under neurogenerative conditions? Or is there a broader role for it in healthy individuals?" she said. "We don't know the answer to those questions yet, and it will be crucial to figure out before pursuing RPS25 as a therapeutic target."

More information: RPS25 is required for efficient RAN translation of C9orf72 and other neurodegenerative disease-associated nucleotide repeats, *Nature Neuroscience* (2019). DOI: 10.1038/s41593-019-0455-7