Potential drug targets for ALS revealed in study using CRISPR

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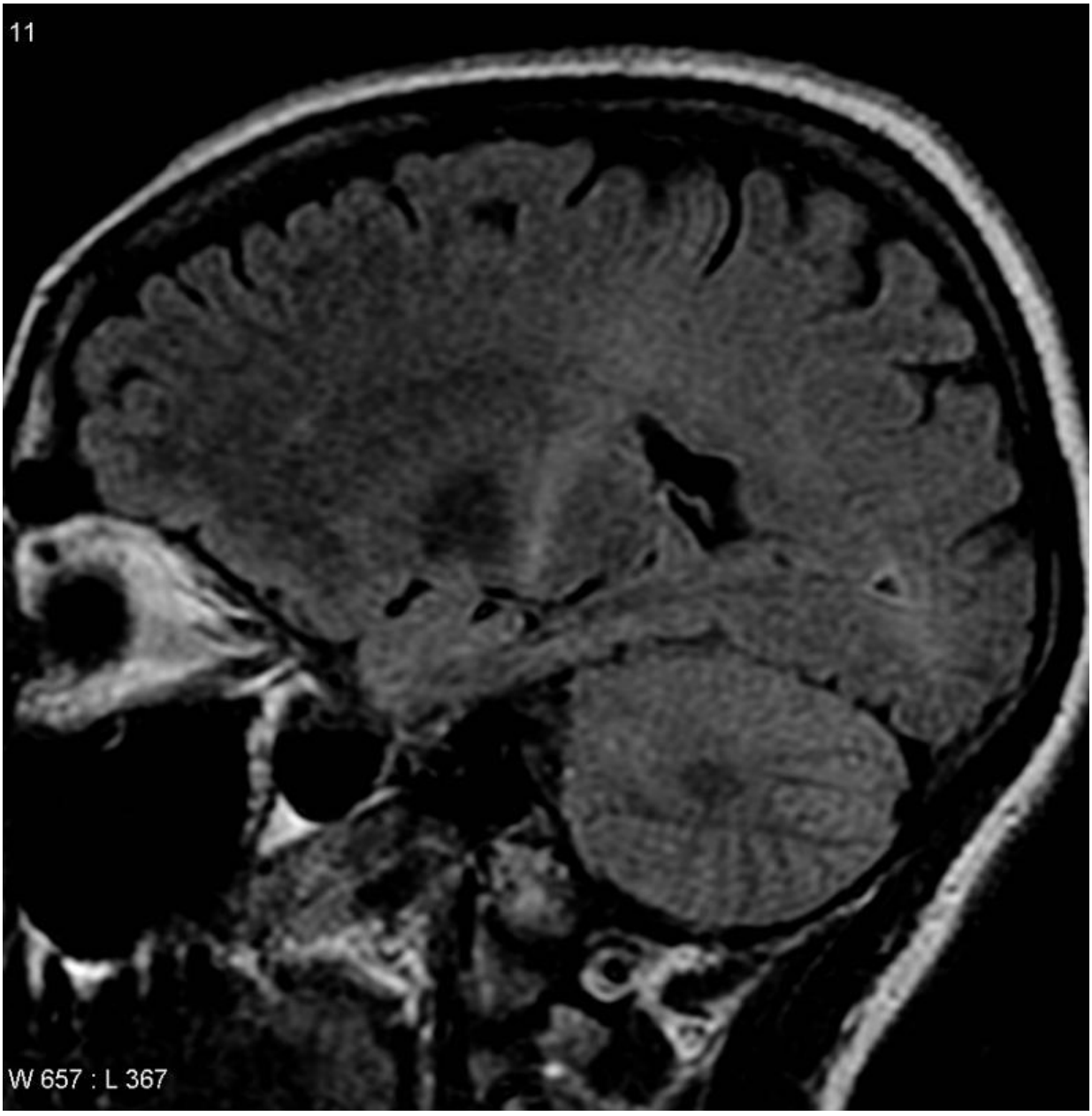

An MRI with increased signal in the posterior part of the internal capsule which can be tracked to the motor cortex consistent with the diagnosis of ALS. Credit: Frank Gaillard/Wikipedia

In a new application of gene-editing technology, researchers at the Stanford University School of Medicine have gleaned insights into the genetic underpinnings of amyotrophic lateral sclerosis, a neurodegenerative disease that's notoriously tricky to parse.

The team's findings are a step toward demystifying how the disease progresses and could even help lay the groundwork for new therapeutic targets.

ALS, also known as Lou Gehrig's disease, erodes muscle function and impairs the brain's ability to communicate with the body, making simple voluntary muscle movements—such as brushing your teeth, talking or even breathing—exceedingly difficult and, eventually, impossible. ALS falls into a category of neurodegenerative diseases that all share a common "signature"—abnormal protein clumps that build up in the brain.

In ALS, these protein clumps, or aggregates, are thought to be fatally toxic to neurons, ultimately leading to the devastating physical symptoms of the disease. But the process of the cells' demise is still largely a black box.

"These toxic protein aggregates are what's likely driving the pathology in the disease, but no one really knows how they cause neuronal cell death. That's really what we wanted to probe in this study," said Aaron Gitler, PhD, professor of genetics. He shares senior authorship with Michael

Bassik, PhD, assistant professor of genetics.

Gitler's and Bassik's labs used CRISPR-Cas9 gene-editing technology to sort through the entire human genome and pick out the genes that helped neurons shore up defenses against the toxic protein. Not only did some genes give the researchers a deeper mechanistic understanding of the disease itself; a handful seem to hold potential as drug targets, too.

A paper describing the research will be published online March 5 in *Nature Genetics*. Graduate students Nicholas Kramer and Michael Haney share lead authorship.

A simple but deadly protein

The discovery that mutations in the C9orf72 gene is a relatively common cause of ALS has helped ignite efforts to understand how ALS works at the molecular level. In ALS, the mutated C9orf72 gene contains a huge segment of DNA that repeats itself and, when that part of the gene is erroneously turned into various rogue proteins, they gum up neuronal function and lead to cell death.

"In a healthy person, you might see 10 to 20 of these DNA repeats," Haney said. "But in ALS, they expand to hundreds or even thousands of repeated segments, and that's the template for the production of these toxic proteins."

Gitler and Bassik set out to answer two basic questions: How do the toxic proteins snuff out otherwise healthy neurons? And are there other genes that inherently protect against—or conversely, exacerbate—the effects of the toxic proteins in the brain?

Rather than separately interrogate every gene in the human repository, the researchers used a tactic called genomewide screening, which

harnesses CRISPR-Cas9 to alter the function of every single human gene simultaneously. In this case, they used the technology to produce "gene knockouts," targeting genes with a kind of molecular scissors that makes precise cuts, leaving them unable to carry out normal function.

The gene knockouts, Kramer explained, help the researchers spot genes that either enhance toxicity or prevent it: If you identify a gene and knock it out, and the ALS protein repeats are no longer toxic, then you know that the absence of that gene actually protects the neuron against degeneration. And perhaps more importantly, it may be a potential drug target.

Tmx2: A sentinel of cell death

After systematically knocking out every gene in the human genome and measuring the toxicity of the ALS proteins in cells, the researchers found that about 200 genes, when knocked out, either helped to protect the cell from the toxic proteins or made it more vulnerable to them. To zero in on a smaller set of genes, Haney and Kramer followed up with two subsequent knockout screens in primary mouse neurons.

They found a handful of knockouts that were particularly potent protectors. One, for example, helped block off critical entrances through which the toxic ALS proteins infiltrate the cell and corrupt it. But there was another knockout in particular that caught the group's attention for its mysterious ability to ward off neural death. The gene normally codes for a protein called Tmx2, which is found in a part of the cell called the endoplasmic reticulum. But when depleted in mouse neurons in a dish, the cells survived nearly 100 percent of the time—quite a jump, considering that the survival rate for normal neurons was 10 percent.

"We could imagine that Tmx2 might make good drug target candidate," Haney said. "If you have a small molecule that could somehow impede

the function of Tmx2, there might be a therapeutic window there."

Right now, Tmx2's role in the endoplasmic reticulum isn't completely clear. But it's thought to be involved in the response to various environmental stressors, particularly those that trigger cell death. According to the study's findings, it may be a modulator of other genes that set off the cell-death process.

"We're still in early phases, but I think figuring out exactly what Tmx2 normally does in a cell is a good place to start—that would hint at what functions are disturbed when these toxic species kill the cell, and it could point to what pathways we should look into," Kramer said.

More broadly, CRISPR screens like the one in this study have been used to investigate a range of disease pathways. But the team said this is the first time, to their knowledge, that a genomewide human CRISPR knockout screen has been used to discover clues about a neurodegenerative disorder. Gitler and Bassik are currently teaming up to use this same approach to understand additional causes of ALS and even other neurological diseases—Huntington's, Parkinson's and Alzheimer's—that involve toxic proteins. "I think it's a really exciting application for CRISPR screens, and this is just the beginning," Bassik said.

More information: CRISPR–Cas9 screens in human cells and primary neurons identify modifiers of C9orf72 dipeptide-repeat-protein toxicity, *Nature Genetics* (2018).
nature.com/articles/doi:10.1038/s41588-018-0070-7

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