New ALS discovery: Scientists reverse protein clumping involved in neurodegenerative conditions

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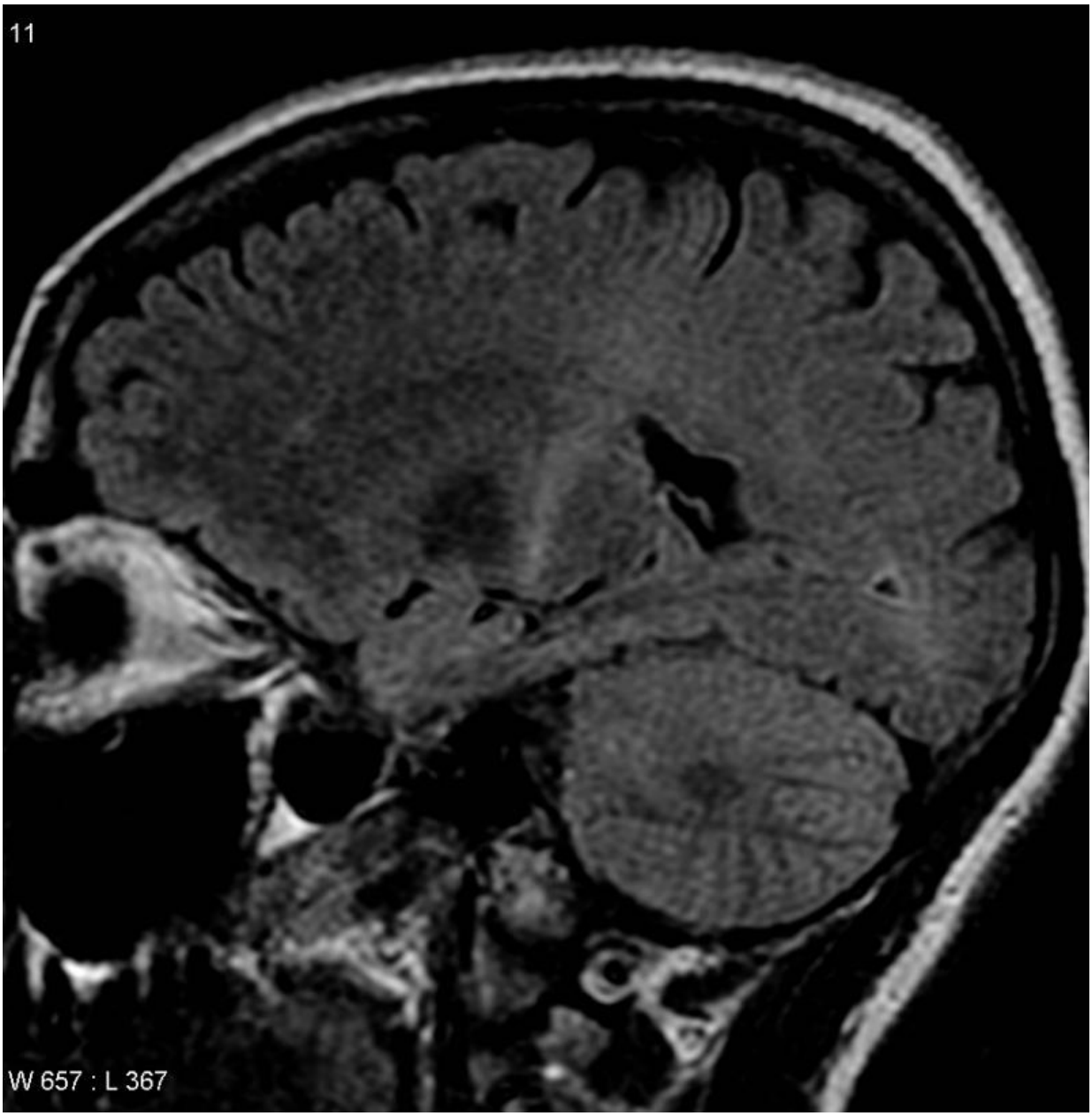

An MRI with increased signal in the posterior part of the internal capsule which can be tracked to the motor cortex consistent with the diagnosis of ALS. Credit: Frank Gaillard/Wikipedia

In the quest to understand the driving forces behind neurodegenerative diseases, researchers in recent years have zeroed in on clumps of

malfunctioning proteins thought to kill neurons in the brain and spinal cord by jamming their cellular machinery. In a new study published in the journal *Structure*, researchers at the UNC School of Medicine announced the first evidence that stabilizing a protein called SOD1 can help reverse this process in the types of neurons affected by the fatal neurodegenerative condition Amyotrophic Lateral Sclerosis (ALS). Also known as Lou Gehrig's disease, ALS has no cure and its causes remain largely mysterious.

In addition to showing that stabilizing SOD1 is protective for motor neuron-like cells, the new study is also the first to demonstrate a way to mutate disease-associated SOD1 in order to stabilize it, offering exciting new leads for finding drugs that could potentially prevent the disease or slow its progression.

"The identified mutation mimics a natural process called phosphorylation, thus suggesting that there may be a natural, or endogenous, mechanism to stabilize SOD1 in cells and prevent the protein from forming toxic oligomers in people without disease," said senior author Nikolay Dokholyan, PhD, the Michael Hooker Distinguished Professor of Biochemistry and Biophysics at UNC. "Understanding the cellular mechanisms resulting in SOD1 phosphorylation not only offers insights about how cells respond to toxic SOD1 clumps, but will potentially offer insights into new pharmaceutical strategies aimed at promoting SOD1 phosphorylation. That is our immediate goal."

Patients with ALS suffer gradual paralysis and early death as a result of the loss of motor neurons, which are crucial to moving, speaking, swallowing, and breathing. In a study published last year, Dokholyan's team discovered that SOD1 forms temporary clumps of three, known as "trimers," and that these clumps are capable of killing motor neuron-like cells grown in the laboratory. When functioning properly, SOD1 exists

in pairs of two, structures known as "dimers."

"The idea was that if we can stabilize SOD1 in the first place, we can potentially provide a way to prevent this disease at an early stage," said Cheng Zhu, PhD, a postdoctoral researcher in Dokholyan's lab and an author of the new study. "Our results here show that stabilizing SOD1 can increase cell viability."

The findings could be particularly relevant to a subset of ALS cases – an estimated 1 to 2 percent – that are associated with variations in the SOD1 protein. However, SOD1 has been implicated in toxic clump formation even in patients without mutations in their SOD1 genes, suggesting that stabilizing the protein could benefit many other patients as well.

Because SOD1 trimers disintegrate almost as soon as they form, the research team first used computational modeling to make educated guesses about what types of modifications might lead SOD1 to clump, or prevent it from doing so. Their models suggested that adding a phosphate group, known as phosphorylation, could help to stabilize the protein. Phosphorylation is known to affect how proteins function in the context of many cellular processes.

To test this hypothesis, the team created a genetic mutation that would mimic the addition of a phosphate group to SOD1 proteins in motor neuron-like cells grown in the lab. The cultured cells they used were developed as part of earlier work to model the mechanics behind ALS and already had one mutation that caused the SOD1 proteins to form toxic clumps.

"When we transfected this new mutation into cells in concert with the disease mutation, it actually rescued toxicity; it made the cells not die," said Jimmy Fay, a graduate student at UNC and former lab technician

who worked on the research. Instead of being killed by toxic clumps of SOD1 the cells survived, thanks to the phosphorylation-mimicking mutation.

The findings offer two new avenues for identifying possible drug targets for treating ALS. One is to find ways to promote phosphorylation of SOD1 in a patient's motor neurons. The other is to look for other ways to stabilize SOD1 where it tends to clump.

"We can now see a way forward," Fay said. "We know that this mutation stabilizes SOD1, and the hope is that we can find a drug that makes the protein act in this way. By slowly piecing together the larger story of how SOD1 acts, hopefully that can be useful in drug studies to try to get a handle on how to affect the behavior of this protein in a planned way."

The findings also may provide clues about why some people get ALS while others do not. If phosphorylation of SOD1 is found to be common in people without ALS, it could indicate that defects leading to reduced phosphorylation play a role in destabilizing SOD1, even in people without detrimental SOD1 mutations.

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