Large-scale generation of muscle-controlling nerve cells from amyotrophic lateral sclerosis patients

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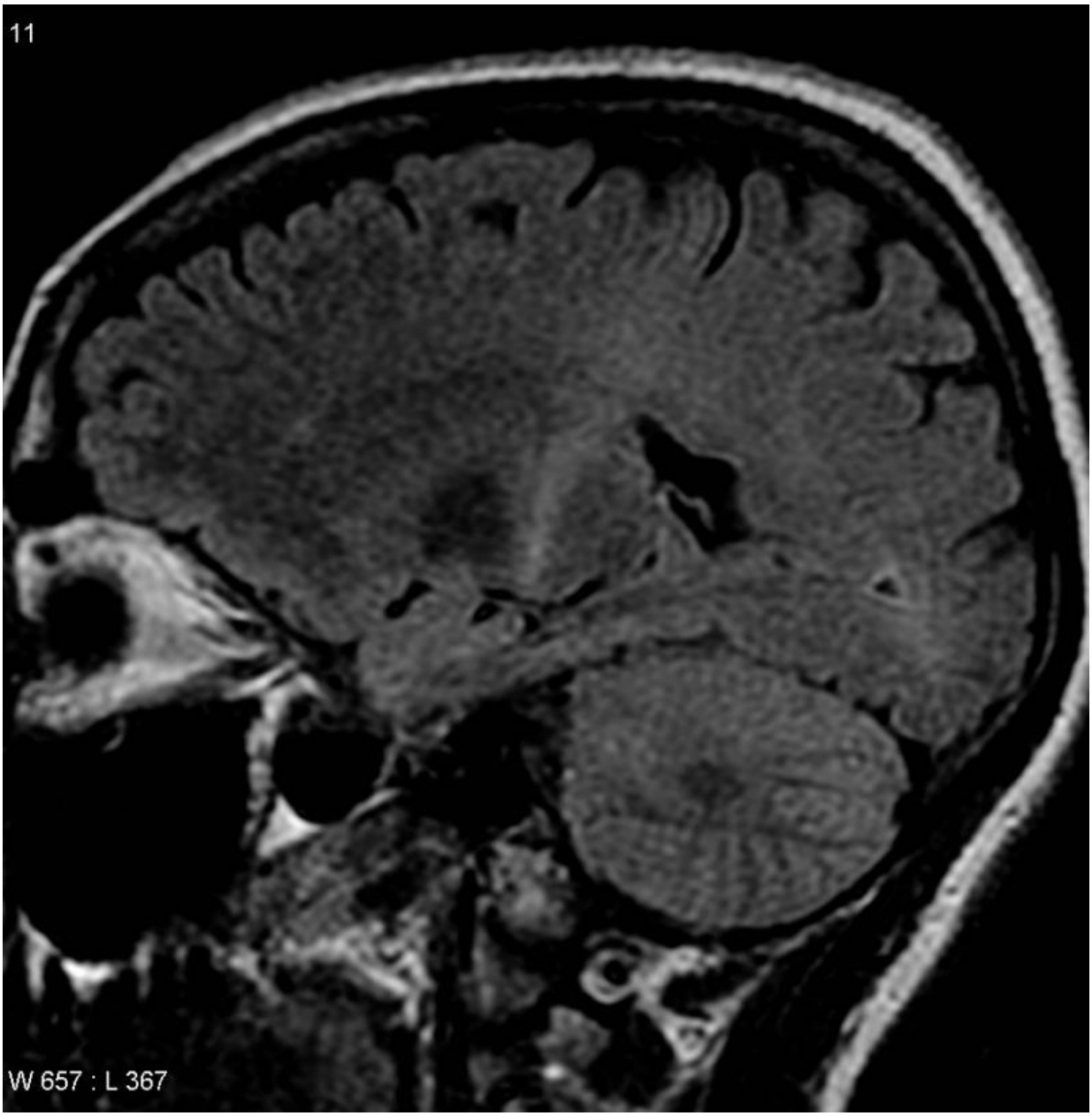

An MRI with increased signal in the posterior part of the internal capsule which can be tracked to the motor cortex consistent with the diagnosis of ALS. Credit: Frank Gaillard/Wikipedia

A new Cedars-Sinai study in collaboration with the University of California, Irvine (UCI) and the Answer ALS consortium has examined

the expression of thousands of genes in stem cell generated motor neurons that are known to die in patients with amyotrophic lateral sclerosis, a fatal neurological disorder known as ALS or Lou Gehrig's disease.

The study, published in the peer-reviewed journal *Neuron*, revealed sex was one of the main drivers of different gene expression in motor neurons, regardless of whether they were from patients diagnosed with ALS.

"This is the first time that nearly 450 lines of stem cells have been simultaneously differentiated and turned into motor neurons from patients with ALS and healthy controls," said Clive Svendsen, Ph.D., executive director of the Cedars-Sinai Board of Governors Regenerative Medicine Institute and professor of Biomedical Sciences and Medicine, who is co-senior and co-corresponding author of the study. "This was a collaborative effort with incredibly hard-working teams of scientists across many laboratories to provide a vital resource for the ALS community."

The discovery is the first set of in-depth findings that stem from Answer ALS, one of the largest resources for ALS biological samples in the world. Answer ALS is part of a collaborative effort with over 100 scientists that includes biological and clinical data from more than 1,000 ALS patients.

The data is generated from patient stem cells that are then turned into motor neurons, the nerve cells responsible for muscle function. These motor neurons give researchers a better understanding of the mechanisms of the fatal disease and can potentially help lead to the development of new therapeutics that target specific cells and pathways.

The samples are available to researchers through the Cedars-Sinai iPSC

Core and Biomanufacturing Center, which is led by Dhruv Sareen, Ph.D., co-corresponding author of the study, the executive director of the Cedars-Sinai Biomanufacturing Center and associate professor of Biomedical Sciences.

While the approach of using human stem cells to model a disease isn't the first, no one has ever analyzed this many stem cell lines from patients with ALS.

ALS is a complex disease that remains poorly understood with no known cure. The disease causes damage to motor neurons in the brain and spinal cord, leading to the loss of muscle control and ultimately movement. Currently, there is no medical treatment that effectively targets the fatal disease, other than measures—like respirators or feeding tubes—to help patients be more comfortable.

To understand why ALS happens and identify distinct molecular signatures of ALS in men and women, the team used 341 stem cell lines from ALS patients, which were differentiated into motor neurons, along with 92 lines from a healthy control group.

Leslie Thompson, Ph.D., co-corresponding author of the study and Bren professor of Psychiatry and Human Behavior and Neurobiology and Behavior at UCI, and her team performed and analyzed RNA sequencing that can measure the expression of up to 32,000 genes in each sample. This gives the teams the ability to detect gene patterns that may be affected by the disease and to see if there were any signals that would separate ALS patients from healthy controls.

Instead of finding prominent differences related to ALS, the team found striking differences between males and females, regardless of whether they were diagnosed with ALS. The investigators also were surprised to find that the male ALS stem cells generated significantly more motor

neurons than the control group, but this was not observed with stem cells from females.

"We hypothesized that we would see differences in gene expression between ALS and healthy control groups, but the changes were subtle" said Michael Workman, Ph.D., a project scientist in the Svendsen Laboratory and co-first author of the study. "However, when we analyzed males and females separately, some specific changes in gene expression were seen—particularly in male ALS samples."

Previous studies using this approach have only used a few lines and often not balanced their experiments for male and female subjects. Only doing this at such a large scale revealed that sex was one of the main drivers of different gene expression in motor neurons.

"It is known that women are less likely to get ALS than men, and if they do get diagnosed with it, it's generally a little later in life and affects different sets of motor neurons," said Svendsen, who is also the Kerry and Simone Vickar Family Foundation Distinguished Chair in Regenerative Medicine. "Now that we have discovered gene expression patterns that can distinguish male and female motor neurons, it may help with designing therapies in the future."

Investigators plan to continue analyzing the data that is continuously being collected and deposited into the Answer ALS online, open-source portal. All of the data described in the resource paper is available for scientists to download, and the stem cells are available through the Cedars-Sinai iPSC Core.

"The take-home message is that these big data sets and large numbers of ALS and control stem cell lines are now available to the ALS community to use and look for new causes and treatments for this devastating disease," Svendsen said.

The study was a collaborative project with Answer ALS and UCI. Dhruv Sareen, Ph.D. and Leslie Thompson Ph.D. were co-senior authors, and Ryan Lim, Ph.D., and Jie Wu, Ph.D., both from UCI, were co-first authors.

More information: Clive N. Svendsen, Large-scale differentiation of iPSC-derived motor neurons from ALS and control subjects, *Neuron* (2023). DOI: 10.1016/j.neuron.2023.01.010. www.cell.com/neuron/fulltext/S0896-6273(23)00034-X

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