Team gains new insight into how motor neurons in the brain die during ALS

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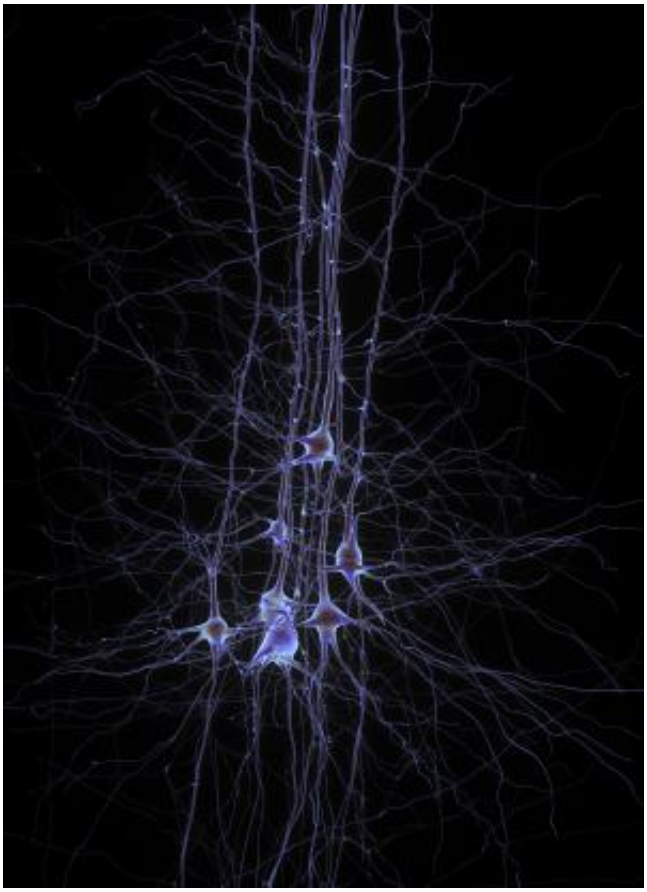

This is a group of neurons. Credit: EPFL/Human Brain Project

Researchers look to understand the causes of amyotrophic lateral sclerosis (ALS), in the hope of finding new ways to treat the disease. A new study published online today in the Cell Press journal *Neuron* shows

that a common gene mutation in ALS generates a deadly protein that may cause the damage in the brain that leads to ALS.

About 5 percent of ALS patients carry an altered version of a gene called C9orf72, which in ALS patients contains hundreds of repeat sequences that otherwise are not present in normal individuals. Since the gene's discovery in 2011, however, researchers have been trying to understand its normal function as well as its role in ALS, with multiple hypotheses proposed.

Senior author Davide Trotti, Ph.D., co-director of the Weinberg Unit for ALS research at Thomas Jefferson University explored three leading hypotheses. The first idea, that the C9orf72 mutation in ALS disrupts the gene's normal function in the cell did not hold up. When the researchers knocked down expression of the gene, reducing how much of the protein was made, neurons continued to behave normally, suggestion that the C9orf72 gene is not essential to neuronal health.

The other possibility was that the repeat sequences contained in this gene generate a product - either RNA or protein - that is toxic to the cell. The RNA transcribed from the C9orf72 gene is folded into an unusual shape called a G-quartet, resembling a stack of plates, which may have interfered with normal cell functions. A third option was that it was that the proteins aberrantly generated from this large repeat sequences in the C9orf72 gene of ALS patients were somehow toxic to neurons.

Dr. Trotti and colleagues generated synthetic version of the RNA G-quartets and inserted them into healthy cells that did not contain the C9orf72 mutation. Neurons that had longer versions of the quartets (more plates in the stack) had two times greater chance of dying than those with fewer G-quartets, suggesting that this mechanism might play a role.

However, the most compelling evidence came when Dr. Trotti and colleagues tested the proteins created from the C9orf72 RNA. Although five distinct proteins could be generated from the same RNA sequence, the researchers found that one of the five caused the greatest amount of damage to the cell. The protein chain made from repeats of the amino acids proline (P) and arginine (R), called a poly-PR chain, accumulated in the nucleolus and very rapidly killed the neuron that produced it.

By tracking the fate of a living neuron in real-time, the researchers could see that as more PR protein accumulated in the nucleolus, the cell became more bloated and then suddenly died. The reaction was rapid, occurring within 72 hours.

To test whether the processes observed in cells held true in humans, the researchers tested iPS cells derived motor neurons from ALS patients who had the C9orf72A mutation for the chains of PR protein. Indeed, the motor neurons harbored the toxic PR protein. In addition when both the RNA chains and the PR proteins were active in the cell, researchers observed a synergistic effect, suggesting both mechanisms may be involved in causing the damage to motor neurons.

"These studies suggest that if we could prevent the formation of PR aggregates or promote breaking them up, we could help prevent the motor neuron damage that causes the symptoms we see in ALS patients," says Dr. Trotti.

More information: X. Wen et al., "Antisense Proline-Arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death," *Neuron*, 2014.

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