

 NEURODEGENERATIVE DISEASE

C9orf72 insufficiency cooperates with toxic repeat peptides to kill neurons

A new study has shed light on how the most common known cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) — a GGGGCC-repeat expansion in *C9orf72* — exerts its toxic effect and leads to neuronal degeneration. Investigators found that a loss of *C9orf72* protein disrupts endosomal trafficking, resulting in an accumulation of glutamate receptors and defective breakdown of toxic dipeptide-repeat (DPR) proteins, both of which promote the death of motor neurons.

The repeat expansion in *C9orf72* causes several problems in cells, including decreased levels of native *C9orf72* and the accumulation of RNA and DPR proteins manufactured from the repeats. However, how these different effects combine to bring about ALS and/or FTD in patients is uncertain, which poses a challenge for the development of therapies for individuals with this mutation.

In the new report, Justin Ichida and colleagues studied induced motor neurons (iMNs) generated from patients with the repeat expansion (patient iMNs) and from healthy

individuals without the expansion (control iMNs). Patient iMNs had lower levels of *C9orf72* than did control iMNs and underwent rapid neurodegeneration when treated with glutamate. The same defect was observed in control iMNs treated with an antisense oligonucleotide against *C9orf72*. The heightened glutamate sensitivity was associated with an increased number of NMDA and AMPA receptors on the cell surface of *C9orf72*-deficient iMNs and was reversed by overexpression of *C9orf72*.

To see how loss of *C9orf72* might exert this excitotoxic effect, the team assessed the location of the protein and found that it colocalizes with markers of early endosomes in control and patient iMNs. However, patient iMNs had fewer lysosomes than did controls, indicating a disruption of lysosome biogenesis in the patients. Conversely, overexpression of *C9orf72* restored the number of lysosomes.

“We wondered whether reduced *C9orf72* activity renders motor neurons vulnerable to other insults that might be exacerbated by endosomal-lysosomal system function, such as

DPR toxicity,” Ichida and colleagues write. When the researchers overexpressed *C9orf72* in patient iMNs, they found that it reduced the number of aggregates that contain the toxic DPR protein proline–arginine.

Interestingly, the team also identified several small molecules that improved the survival of patient iMNs by modulating endosomal trafficking pathways, suggesting that these processes might be a therapeutic target in individuals with ALS or FTD who have the *C9orf72* repeat expansion.

The investigators claim that their findings highlight the relevance of both loss-of-function and gain-of-function mechanisms of pathogenesis in patients with the *C9orf72* repeat expansion. “Our results establish a new approach for suppressing DPR protein toxicity and blocking *C9orf72* pathogenesis: restoring or replacing *C9orf72* activity,” Ichida and colleagues write.

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ORIGINAL ARTICLE Shi, Y. et al. Haploinsufficiency leads to neurodegeneration in *C9ORF72* ALS/FTD human induced motor neurons. *Nat. Med.* <https://doi.org/10.1038/nm.4490> (2018)