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Targeting RNA Foci in iPSC-Derived Motor Neurons from ALS Patients with a C9ORF72 Repeat Expansion.

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Public Summary:
Amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) is a uniformly fatal disease caused by the death of cells in the nervous system that control the musculature. Patients slowly become paralyzed and lose the ability to breathe, and no effective therapies currently exist. The expansion of a repeated DNA element (GGGGCC) in a gene called C9ORF72 was recently identified as the most common genetic cause of ALS. In this study, we set out to understand how the expansion of the GGGGCC repeat in C9ORF72 causes cell degeneration. To do this, skin cells from patients with the disease were converted into motor neurons (the cells that die in ALS) in a culture dish. They found that large pieces of RNA containing the expanded GGGGCC repeat built up in neurons from ALS patients and disrupted the function of these cells. Furthermore, we observed that oligonucleotides complementary to the C9ORF72 RNA transcript sequence ("antisense oligonucleotides") suppressed the formation of these RNA foci. These findings support the idea that the buildup of "toxic" RNA containing the GGGGCC repeat contributes to the death of motor neurons in ALS, and suggest that antisense oligonucleotides targeting this transcript may be a strategy for treating ALS patients with the C9ORF72 repeat expansion.

Scientific Abstract:
Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative condition characterized by loss of motor neurons in the brain and spinal cord. Expansions of a hexanucleotide repeat (GGGGCC) in the noncoding region of the C9ORF72 gene are the most common cause of the familial form of ALS (C9-ALS), as well as frontotemporal lobar degeneration and other neurological diseases. How the repeat expansion causes disease remains unclear, with both loss of function (haploinsufficiency) and gain of function (either toxic RNA or protein products) proposed. We report a cellular model of C9-ALS with motor neurons differentiated from induced pluripotent stem cells (iPSCs) derived from ALS patients carrying the C9ORF72 repeat expansion. No significant loss of C9ORF72 expression was observed, and knockdown of the transcript was not toxic to cultured human motor neurons. Transcription of the repeat was increased, leading to accumulation of GGGGCC repeat-containing RNA foci selectively in C9-ALS iPSC-derived motor neurons. Repeat-containing RNA foci colocalized with hnRNPA1 and Pur-alpha, suggesting that they may be able to alter RNA metabolism. C9-ALS motor neurons showed altered expression of genes involved in membrane excitability including DPP6, and demonstrated a diminished capacity to fire continuous spikes upon depolarization compared to control motor neurons. Antisense oligonucleotides targeting the C9ORF72 transcript suppressed RNA foci formation and reversed gene expression alterations in C9-ALS motor neurons. These data show that patient-derived motor neurons can be used to delineate pathogenic events in ALS.