N-terminal truncation of TDP-43 drives splicing deficits and synergizes with C9orf72 in a mouse model of ALS/FTD

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are characterized by the nuclear depletion and cytoplasmic aggregation of TAR DNA-binding protein-43 (TDP-43). TDP-43 dysfunction is a pathological hallmark of approximately 97% of ALS and 50% of FTD cases, resulting in loss of its nuclear function, impaired RNA processing, and the accumulation of cryptic RNAs. While ALS/FTD is a diverse spectrum of disease with many overlapping and distinct underlying mechanisms, the most common genetic cause of ALS/FTD is the presence of hexanucleotide repeat expansions (HREs) in chromosome 9 open reading frame 72 (*C9orf72*). In *C9orf72*-ALS/FTD, repeat-associated non-AUG (RAN) translation of these HREs generates toxic dipeptide repeat proteins (DPRs), which often colocalize with hyperphosphorylated cytoplasmic TDP-43 aggregates, suggesting a pathological synergy. We generated a knock-in *Tdp-43* mouse model utilizing CRISPR-mediated genome editing to delete the extreme N-terminal amino acids 2-9 (Tdp-$43_{\Delta 2\text{-}9}$), disrupting TDP-43 stability, homodimerization, and splicing function, promoting its mislocalization. To evaluate the synergistic toxicity of TDP-43 dysfunction and DPRs, we injected neonatal Tdp-$43_{\Delta 2\text{-}9}$ pups (P0) intracerebroventricularly (i.c.v.) with adeno-associated virus expressing 149 *C9orf72* HRE repeats (C9orf72-149R). Tdp-$43_{\Delta 2\text{-}9}$ mice demonstrated impaired RNA splicing by 12 months of age, although no overt behavioral deficits were observed. In contrast, Tdp-$43_{\Delta 2\text{-}9}$ mice injected with *C9orf72*-149R exhibited lower grip strength, increased pTDP-43 and DPR burden, and increased *C9orf72* expression at 6 months of age. Strikingly, these neuropathological hallmarks and behavioral deficits were accompanied by elevated neurofilament light chain (NfL) levels, indicating neurodegeneration. Together, these findings support a convergent two-hit mechanism underlying ALS/FTD pathogenesis.

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