

Mutant allele-specific CRISPR disruption in DYT1 dystonia fibroblasts restores cell function

ABSTRACT

Most individuals affected with DYT1 dystonia have a heterozygous 3-bp deletion in the TOR1A gene (c.907_909delGAG). The mutation appears to act through a dominant-negative mechanism compromising normal torsinA function, and it is proposed that reducing mutant torsinA may normalize torsinA activity. In this study, we used an engineered Cas9 variant from *Streptococcus pyogenes* (SpCas9-VRQR) to target the mutation in the TOR1A gene in order to disrupt mutant torsinA in DYT1 patient fibroblasts. Selective targeting of the DYT1 allele was highly efficient with most common non-homologous end joining (NHEJ) edits, leading to a predicted premature stop codon with loss of the torsinA C terminus (delta 302–332 aa). Structural analysis predicted a functionally inactive status of this truncated torsinA due to the loss of residues associated with ATPase activity and binding to LULL1. Immunoblotting showed a reduction of the torsinA protein level in Cas9-edited DYT1 fibroblasts, and a functional assay using HSV infection indicated a phenotypic recovery toward that observed in control fibroblasts. These findings suggest that the selective disruption of the mutant TOR1A allele using CRISPR-Cas9 inactivates mutant torsinA, allowing the remaining wild-type torsinA to exert normal function.

Keyword: CRISPR; Dystonia; DYT1; TorsinA; Herpes simplex virus type 1; TOR1A; Nuclear envelope; Neurologic; Hereditary; Gene therapy