Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease that causes progressive loss of motor coordination, respiratory issues and eventual death. SCA1 is caused by expansion of the polyglutamine repeat region in the ATXN1 gene. Normal ATXN1 alleles contain 6-42 CAG trinucleotide repeats with interspersed CAT nucleotides, while disease alleles have an uninterrupted CAG region with 39-100+ repeats. The mechanism of SCA1 pathogenesis is unknown; however, some features of the disease include neuronal degeneration and formation of toxic mutant ATXN1 (mATXN1) nuclear inclusions. Although mATXN1 is expressed ubiquitously, it affects primarily Purkinje cells (PCs). There are currently no treatment options for SCA1. We hypothesize that CRISPR-Cas editing of ATXN1 will reduce mutant ATXN1 and be therapeutically beneficial.

CRISPR Cas9 is a DNA editing tool used to induce knockout of the target gene. For Cas9, we designed two different strategies to reduce ATXN1; the first uses a single guide RNA (gRNA) to target near the exon-exon junction to induce nonsense mediated decay, while the second approach employs a dual guide system to delete the CAG repeat region. gRNAs were optimized in vitro, with each approach significantly reducing ATXN1 expression. The single guide approach reduced ATXN1 mRNA levels by 40-45% (p≤0.02) and protein by approximately 20% (p≤0.01) and the dual guide approach reduced levels of mRNA and protein levels by 70-75% (p<0.001) and 45-65% (p≤0.03), respectively.

For testing in vivo, SCA1 mice, expressing mutant human ATXN1, were crossed to spCas9 transgenic mice. Recombinant AAVs (rAAVs) expressing the optimized gRNAs from each strategy were delivered directly to the deep cerebellar nuclei of 5-week-old SCA1/spCas9 mice for transduction of Purkinje cells. The exon-exon strategy reduced protein and mRNA levels by 55% (p=0.05) and 50% (p=0.02), respectively compared to saline injected controls. The dual guide strategy reduced ATXN1 mRNA levels by 70-80% (p<0.001). This shows that the dual guide strategy is more effective at inducing reduction of ATXN1 levels in vivo and studies are in progress to assess the impact of both strategies on SCA1 mice phenotypes.

In a third strategy, we assessed the utility of CasRx, an RNA specific nuclease which binds to target mRNA and cleaves the sequence complementary to the gRNA. CasRx guides designed to target ATXN1 transcripts reduced ATXN1 mRNA by 50% (p<0.001) in vitro. gRNAs were then delivered via rAAV to the cerebellum of SCA1 mice and the treated cerebellum was collected and analyzed. In vivo CasRx resulted in a 10% ATXN1 mRNA reduction (p=0.001). Currently, we are designing and screening a multiple guide strategy to increase ATXN1 knockdown. This work indicates several editing strategies to treat SCA1 in mice models with possible translation to human therapies.