Lowering the pathogenic exon 1 HTT fragment by AAV5-miHTT gene therapy
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**BACKGROUND**
Huntington disease (HD) is a fatal neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the huntingtin (HTT) gene. This mutation is translated into a polyQ tract in the HTT protein which confers toxicity. Recently, it has been demonstrated that, apart from the full-length mutant HTT (mHTT) protein, exon 1 HTT fragments generated by aberrant splicing of intron 1 are prone to aggregate and contribute to HD pathology.1,2 These findings suggest that approaches capable of reducing the expression of the highly pathogenic exon 1 HTT protein might achieve a greater therapeutic benefit than only targeting the full-length mHTT protein.

We have developed an engineered microRNA targeting exon 1 HTT (mHTT), delivered via adeno-associated serotype 5 virus (AAV5).3 AAV5-miHTT treatment has been demonstrated to lower mHTT in several rodent models (including the exon 1 R6/2 mouse model) and large animal models.4-6

**OBJECTIVES**
Here, we investigated the efficacy of AAV5-miHTT to reduce the aberrantly spliced exon 1 HTT mRNA fragment in knock-in HD mice.

- To confirm the presence of aberrantly spliced exon 1 HTT mRNA transcripts in Q175 KI mice.
- To investigate the lowering of exon 1 HTT mRNA by AAV5-miHTT treatment in Q175 KI mice.

**METHODS**
Q175FDN mice
The Q175FDN mouse model is an enhanced HD knock-in model containing human exon 1 HTT sequence (175 CAG repeats) in the HTT mouse homolog. Removal of the neomycin cassette results in increased mHTT expression, earlier symptom onset and a severe HD-like phenotype.7 Homozygous Q175FDN mice at three months of age were treated by bilateral intrastriatal injection with two doses of AAV5-miHTT or formulation buffer.

**RESULTS**
Successful detection of aberrantly spliced exon 1 mRNA transcript in Q175FDN mice
- Short exon 1 HTT mRNA transcripts were detected in striatum and cortex of Q175FDN mice, but not in WT mice by 3’RACE-PCR (Fig. 3A) and RT-PCR (Fig. 3B).

One-time intrastratal injection results in brain distribution and expression of mHTT
- Three months after AAV5-miHTT treatment, AAV5 dose-dependent levels of vector DNA and mature mHTT molecules were detected in the striatum (injection site) and cortex of Q175FDN mice (Fig. 4A and B).

**CONCLUSIONS**
- Successful detection of aberrantly spliced exon 1 mRNA in striatum and cortex of HD Q175FDN mice.
- Widespread distribution of therapeutic miHTT in striatum and cortex in Q175FDN mice.
- AAV5-miHTT treatment demonstrated lowering of full-length HTT mRNA in striatum and cortex.
- Significant lowering of exon 1 mRNA transcript after AAV5-miHTT treatment.

The successful lowering of pathogenic exon 1 HTT fragment adds therapeutic value to AAV5-miHTT gene therapy for HD.

**REFERENCES**

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