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

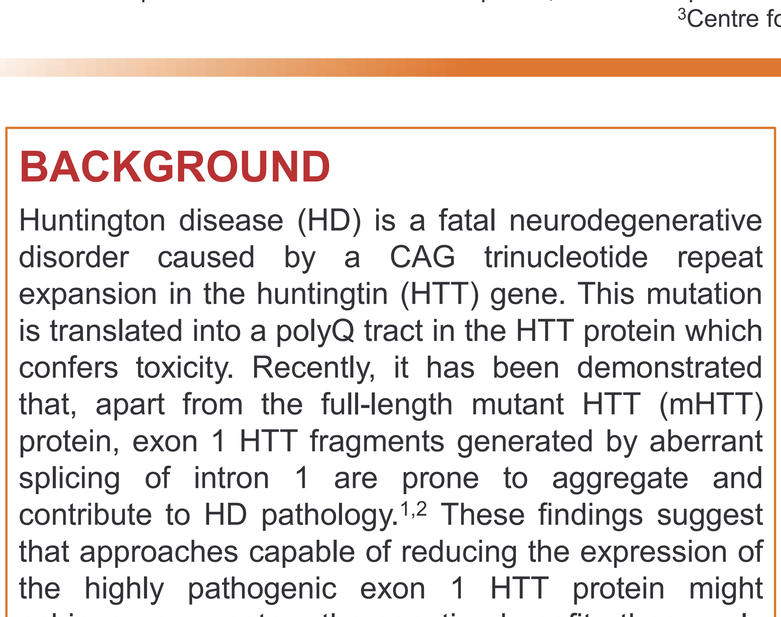

Figure 1. Targeting exon 1 HTT by AAV5-miHTT

OBJECTIVES

Here, we investigated the efficacy of AAV5-miHTT to reduce the aberrantly spliced exon 1 HTT 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.

Table 1. Q175 mice and doses

Strain	Treatment	Dose
Wild type (WT)	Formulation buffer	
Q175FDN	Formulation buffer	
Q175FDN	AAV5-miHTT	Low dose ($5.2x10^9$ gc/mouse) High dose ($1.3x10^{11}$ gc/mouse)

Brain sampling overview

3'RACE-PCR to detect exon 1 HTT mRNA

- Polyadenylated exon 1 HTT mRNA was selectively detected by 3' RACE using primers targeting intron 1 sequence.

Figure 2. 3'RACE. *Oligo-dT primers were used to reverse transcribed polyadenylated mRNA. Primers targeting intron 1 were used to selectively amplified aberrantly spliced exon 1 HTT mRNA transcript, but not full-length HTT.[1,2] RACE, Rapid Amplification of cDNA Ends.*

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**).
- Expression levels of full-length HTT mRNA ("exon 1-2", "exon 2") were downregulated in Q175FDN mice (**Fig. 3C**), while higher expression of exon 1 HTT mRNA ("intron 1") was detected in Q175FDN compared to WT (**Fig. 3D**).

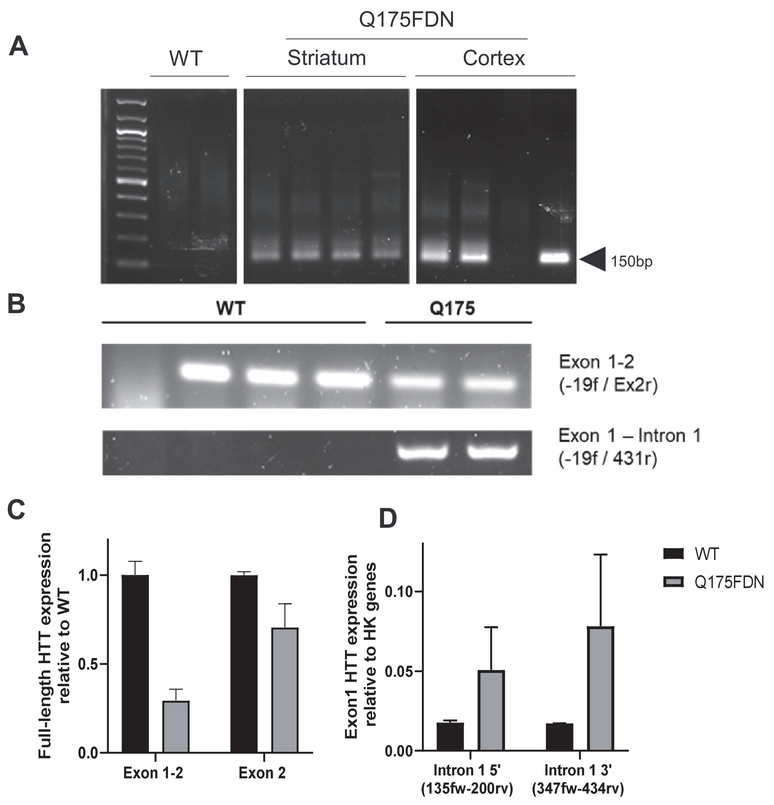

Figure 3. Detection of exon 1 HTT mRNA in Q175FDN mice. *(A) 3'RACE-PCR and (B) RT-PCR. (C) R*elative expression of full-length and exon 1 HTT mRNA was quantified by RT-qPCR in Q175FDN compared to WT mice.

One-time intrastriatal injection results in brain distribution and expression of miHTT

- Three months after AAV5-miHTT treatment, AAV5 dose-dependent levels of vector DNA and mature miHTT molecules were detected in the striatum (injection site) and cortex of Q175FDN mice (**Fig. 4A and B**).

Figure 4. (A) AAV5-miHTT vector DNA distribution and (B) miHTT expression in striatum (left) and cortex (right) at three months after striatal Injection in Q175FDN mice. *Levels of viral DNA (gc/µg DNA) and miHTT expression (molecules/cell) were quantified by TaqMan RT-qPCR (mean ± SEM).*

AAV5-miHTT treatment lowers full-length and exon 1 HTT mRNA in Q175FDN mice

- Significant dose-dependent reduction of full-length HTT mRNA was measured in striatum after AAV5-miHTT treatment (**Fig. 5A**).
- Significant lowering of full-length HTT mRNA was observed in cortex after high dose AAV5-miHTT treatment (**Fig. 5B**).

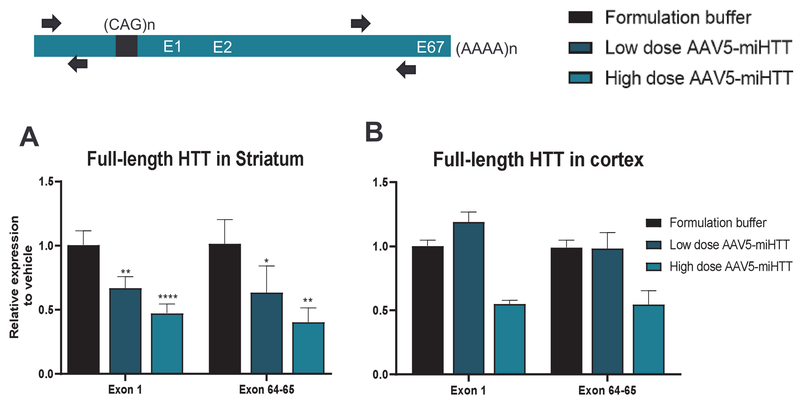

Figure 5. Lowering of full-length HTT mRNA in Q175FDN mice. *(A) Relative expression of HTT mRNA in striatum and (B) in cortex was quantified by RT-qPCR with specific primers targeting "exon 1" and "exon 64-65" (mean ± SEM). Data were analyzed by one-way ANOVA (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).*

- AAV5-miHTT treatment resulted in significant dose-dependent lowering of aberrantly spliced exon 1 HTT mRNA in striatum in Q175FDN mice (**Fig. 6A**).
- Lowering of exon 1 HTT mRNA was observed in cortex in Q175FDN mice after high dose treatment with AAV5-miHTT (**Fig. 6B**)

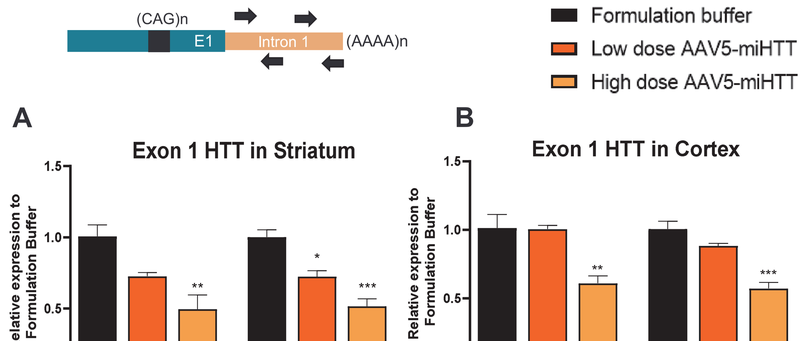

Figure 6. Lowering of exon 1 HTT mRNA in Q175FDN mice. *(A) Relative expression of exon 1 transcript in striatum and (B) in cortex was quantified by RT-qPCR with specific primers targeting "Intron1 5' " and "Intron1 3' " (mean ± SEM). Data were analyzed by one-way ANOVA (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).*

CONCLUSIONS

- Successful detection of aberrantly spliced exon 1 HTT 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.

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