Exploring the effects of intrastratal AAV5-miHTT therapy on biomarker levels in the Q175FDN mouse model of Huntington's disease

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BACKGROUND

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the HTT gene that encodes a polyglutamine expansion in the huntingtin protein (HTT). The primary neuropathological phenotype of HD is selective loss of striatum spiny neurons (SNs) of the striatum, which is accompanied by psychological changes, loss of motor coordination, and intracellular Mislocalization and degradation. HD (HTT) forms toxic nuclear and cytoplasmic aggregates that disrupt multiple aspects of cellular homeostasis, including gene expression. The expanded polyQ region of HD pathologically interacts with regulatory regions of promoters and disrupts transcription factor binding. The HTT polyQ tract also interacts with polyQ functional domains in other cellular proteins (including TFs), sequesters them within miHTT aggregates, and impedes their activity. Reduction of miHTT in mouse models of HD improves multiple aspects of HD pathophysiology, most of which are assessed post-mortem. Minimally invasive translational efficacy measures are therefore crucial for the assessment of HTT-lowering therapies in HD patients in vivo. We explored the use of a non-invasive technique, magnetic resonance spectroscopy (MRS), as a possible means to measure the effects of HTT lowering using an adeno-associated virus serotype 5, engineered microRNA vector targeting human HTT (AAV5-miHTT). These month-old homozygous Q175FDN HD mice were injected bilaterally into the striatum with formulation buffer (sham), low (0.2 X 10^9 g/ml) or high (1.3 X 10^9 g/ml) doses of AAV5-miHTT, followed by a blood draw 3 months post-infusion. We injected Q175FDN mice in two cohorts: one received a high dose (2.6 X 10^11 g/ml) and the second received a lower dose (4.0 X 10^10 g/ml) for an extended period of 12 months. We evaluated the neurochemical profile of HD mice treated with different doses of AAV5-miHTT using 1H-MRS. The authors would like to thank Dr. Nicholas Caron for performing statistical analysis of the data.

METHODS

Mechanism of action (MoA) of AAV5-miHTT

1. Upon parenchymal injection, AAV5-miHTT binds to neuronal cell surface receptors and is internalized.
2. AAV5-miHTT is transported to the nucleus. The miHTT transgene is excised and transcribed post-translational.
3. Expression and processing of the miHTT transgene is performed by endogenous enzymes.
4. The multiple-stranded precursor is processed and further processed to form mature guide miHTT. This 40 nt RNA strand weakly binds to the miHTT.
5. Mutant huntingtin gene is knocked down in a dosedependent manner, resulting in lowering of huntington protein translation.

Stereotaxic injections of AAV into Q175FDN mouse striatum: Three month-old homozygous Q175FDN mice were stereotaxically injected bilaterally into the striatum with formulation buffer (sham), low dose (0.2 X 10^9 g/ml) or high dose (1.3 X 10^9 g/ml) AAV5-miHTT.

AAV distribution in the CNS: HD transgenic mouse received bilateral stereotaxic injections at two months of age with an AAV5 vector expressing green fluorescent protein (AAV5-GFP). At the end of the experiment, mice were sacrificed for histological analysis. AAV vector expression was validated by confocal imaging of GFP-labeled neurons in the striatum. The distribution of AAV within the brain: HD mice received bilateral stereotaxic injections with a scramble miRNA integrated at the injection site. The authors would like to thank Dr. Nicholas Caron for performing statistical analysis of the data.

RESULTS

In vivo assessment of HD pathophysiology in response to AAV5-miHTT therapy in Q175FDN mice

Brain distribution and target engagement in response to AAV5-miHTT therapy in Q175FDN mice

Figure 1. AAV5-miHTT infusion resulted in measurable, significant levels of vector DNA and miHTT in relevant brain regions such as striatum and cortex, with corresponding decreased miHTT protein levels. As expected, no significant levels were detected in cerebellum except for minor effects with high dose.

Differential gene expression levels in response to AAV5-miHTT therapy in Q175FDN mice

Figure 4. Heat map of fold change of the top 40 differentially expressed genes between WT PBS and Q175FDN PBS treatment groups. There is a dose-dependent shift of HTT expression levels towards WT expression levels.

SUMMARY OF RESULTS

- Significant levels of viral DNA were present in treated tissue, which resulted in robust expression of miHTT
- There was significant knockdown of mutant huntingtin protein following administration of AAV5-miHTT in Q175FDN mice.
- AAV5-miHTT infusion did not have a significant effect on miHTT or NfL protein levels in blood plasma.
- Q175FDN mice showed robust regional volume loss in multiple brain regions at 6 months of age, measured using structural MRI. High dose AAV5-miHTT infusion significantly prevented hippocampal volume loss.
- There was significant preservation of NfA4 levels following 3 months of treatment with high dose AAV5-miHTT.
- Transcriptomic analysis shows significant AAV5-miHTT dose-dependent HTT reduction and suggests HTT knockdown directly impacts miHTT-associated transcriptional dysregulation.

FUTURE DIRECTIONS

- Additional transcriptomic analysis will be performed to identify functional significance of differentially expressed genes in response to the AAV5-miHTT-induced reduction of miHTT.
- DEGs with putative functional significance will be validated in independent biological replicate samples using RT-qPCR.

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