Exploring the effects of intrastratal AAV5-miHTT lowering therapy on MRS signal and mutant huntingtin levels in the Q175FDN mouse model of HD
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**BACKGROUND**

Translational efficacy measures are crucial for the assessment of HTT-lowering therapies in HD patients. We explored the use of a non-invasive technique, magnetic resonance spectroscopy (MRS), as a potential in vivo measure of the effects of HTT lowering using an adeno-associated virus serotype 5, engineered microRNA vector targeting human HTT (AAV5-miHTT). Three-month-old homozygous Q175FDN HD mice were injected bilaterally into the striatum with formulation buffer ( sham), low (5.2 X 10^8 gc/mouse) or high (1.3 X 10^9 gc/mouse) doses of AAV5-miHTT. Wild-type (WT) mice injected with formulation buffer served as controls. 11^th}-weighted structural MRI imaging (MRI) and striatal MRI were performed 3 months after injection, and shortly afterwards the animals were sacrificed to collect brain tissue. Decreased total N-acetylaspataate (NAA), neuronal integrity marker) and increased myo-inositol (MI, glial marker) levels were found in Q175FDN sham-treated mice with respect to WT controls, similarly to previous observations in the putamen of HD patients. These findings were reversed in the Q175FDN high-dose AAV5-miHTT treated mice with higher levels of NAA and reduced levels of MI compared to sham-treated Q175FDN mice. Structural MRI showed reduced volumes in stratum, cortex, hippocampus and thalamus of Q175FDN mice versus WT controls, with partial reversal of hippocampal volume loss in the high-dose AAV5-miHTT treated mice. Dose-dependent changes in AAVS vector DNA level, mHTT expression and HTT protein were observed in stratum and cortex. Correlations were shown between NAA MRI levels and AAV5 vector DNA, mHTT and HTT protein levels in stratum and cortex, suggesting a direct relationship between our AAVS-miHTT therapy, HTT lowering and the striatal MRS signal. Stratal MRI analysis suggests a restoration of neuronal function and partial reversal of gliosis after AAV5-miHTT treatment, strengthening the therapeutic potential of AAVS-miHTT in lowering HTT and reversing the neuropathology of HD, and supporting the use of MRS for HTT-lowering clinical trials in HD.

**METHODS**

**Mechanism-of-action (MoA) of AAV5-miHTT**

1. Upon parentrmary injection AAV5-miHTT binds to neuronal surface receptors and is internalized.
2. Transport to the nucleus and uncoiling of the mRNA transcript which remains mainly unprocessed.
3. Expression and processing of the mature miRNA transcript by the endogenous RNA interference machinery.
4. Hexon structural protein is cleaved off to the miRNA and further processed to mature guide miRNA.
5. Non-parentrmary injection in the striatum and further processed to mature guide miRNA.
6. Expression and processing of the mature miRNA transcript by the endogenous RNA interference machinery.
7. miRNA binds to its target and is cleaved and degraded, resulting in lowering of huntingtin protein translation.

**RESULTS**

**Behavioural Measures**

A. **Accelerating Rotatord**

B. **Novel Object Recognition**

**Plasma Biochemistry Measures**

**Brain Biochemistry Measures**

C. **miHTT Protein**

D. **NF-L Protein**

E. **Striatum**

F. **Cortex**

G. **Cerebellum**

**Structural Brain MRI Measures**

A. **Striatal Volume**

B. **Cortical Volume**

C. **Hippocampal Volume**

D. **Thalamic Volume**

**Striatal MRS Measures**

**Figure 1.** Behavioural, Plasma Biochemical, and Brain Biochemical measures in response to AAV5-miHTT therapy in Q175FDN mice. Q175FDN mice have deficits in rotarod performance (A) but not novel object recognition (B) compared to wild-type mice. Neurofilament light chain protein (D) but not miHTT (C) was elevated in Q175FDN plasma mice as well. AAVS-miHTT infusion had no significant effect on any of these measures, but we could measure significant levels of vector DNA and HTT protein in brain with decreased miHTT levels.

**Figure 2.** Structural MRI volume measures in response to AAV5-miHTT infusion in Q175FDN mice. Compared to wild-type mice, Q175FDN mice had volume loss in the Striatum (A), Cortex (B), Hippocampus (C), and Thalamus (D). Intrastrial infusion of AAVS-miHTT did not significantly reverse this volume loss except for an effect of the highest dose in Hippocampus (*p < 0.05)

**Figure 3.** MRS changes in the striatum in response to AAV5-miHTT infusions in Q175FDN mice. Q175FDN mice have decreased striatal levels of the neuronal marker tNAA and elevated levels of the glial marker MI similar to human HD. Treatment with AAVS-miHTT significantly reversed the changes in tNAA at the highest doses tested.

**Correlation of mHTT with MRS Measures**

**Figure 5.** Correlation of striatal mutant huntingtin levels with tNAA and MI in Q175FDN mice following in AAVS-miHTT infusions. There was a significant correlation between decreased levels of mHTT expression in the striatum of Q175FDN mice and higher levels of tNAA and lower levels of MI.

**SUMMARY OF RESULTS**

- Intra-striatal administration of AAVS-miHTT had no significant impact on behavioural deficits when administered at 2-3 months of age in the Q175 mouse model of HD.
- Vector analysis showed significant levels of virus present in treated tissue which resulted in robust expression of miHTT.
- There was significant knock-down of mutant huntingtin following administration of AAVS-miHTT in Q175FDN mice.
- There is a significant restoration of fMRI levels following 2 months of treatment with AAVS-miHTT at the highest dose tested.

**CONCLUSIONS**

- Q175FDN mice are an appropriate HD mouse model for studying MRS and MRI related brain changes.
- Delivery of AAVS-miHTT at 2-3 months of age lead to significant improvements in brain metabolites as measured by fMRI.
- Silencing of mHTT in the brain continues to be a viable therapeutic strategy.
- Final point?

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