Centre for Molecular Medicine and Therapeutics



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

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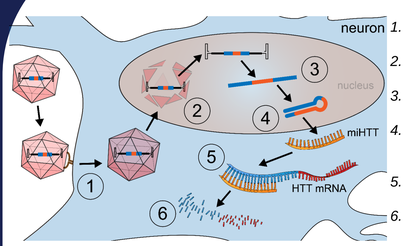
BACKGROUND

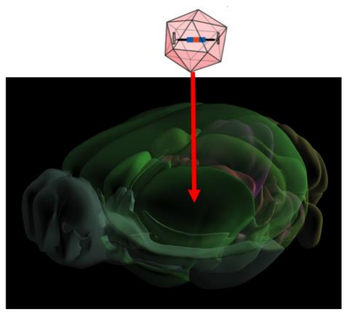
Translational efficacy measures are crucial for the assessment of HTT-lowering therapies in HD patients. We explored the use of a non-invasive technique, magnetic resonance spectroscopy (MRS), as a potential in vivo measure of the effects of HTT lowering using an adeno-associated virus serotype 5, engineered 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 10^9 gc/mouse) or high (1.3 X 10^{11} gc/mouse) doses of AAV5-miHTT. Wild-type (WT) mice injected with formulation buffer served as controls. T1-weighted structural MR imaging (MRI) and striatal MRS were performed 3 months after injection, and shortly afterwards the animals were sacrificed to collect brain tissue. Decreased total N-acetylaspartate (tNAA, neuronal integrity marker) and increased myo-inositol (MI, gliosis 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 tNAA and reduced levels of MI compared to sham-treated Q175FDN mice. Structural MRI showed reduced volumes in striatum, 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 AAV5 vector DNA level, miHTT expression and HTT protein were observed in striatum and cortex. Correlations were shown between tNAA MRS levels and AAV5 vector DNA, miHTT and HTT protein levels in striatum and cortex, suggesting a direct relationship between our AAV5-miHTT therapy, HTT lowering and the striatal MRS signal. Striatal MRS analysis suggests a restoration of neuronal function and partial reversal of gliosis after AAV5-miHTT treatment, strengthening the therapeutic potential of AAV5-miHTT in lowering HTT and reversing the neuropathology of HD, and supporting the use of MRS for HTT-lowering clinical trials in HD.

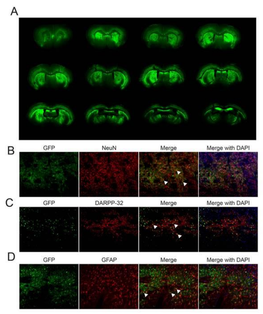
METHODS

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



1. *Upon parenchymal injection AAV5-miHTT binds to neuronal cell-surface receptors and is internalized.*
2. *Transport to the nucleus and uncoating of the miHTT transgene which remains mostly episomal.*
3. *Expression and processing of the miHTT transgene by the endogenous RNA interference machinery.*
4. *Hairpin structured precursor is transported to the cytoplasm and further processed to mature guide miHTT. No passenger strand is formed, strongly limiting the risk of off-target activity.*
5. *Mature miHTT is loaded in the RNA-induced silencing complex and binds HTT mRNA.*
6. *HTT mRNA is cleaved and degraded, resulting in lowering of huntingtin protein translation.*

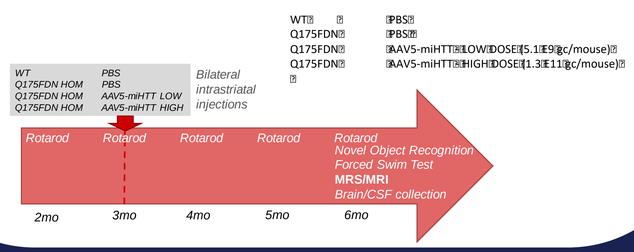




Stereotaxic injections of AAV5 into Q175FDN Mouse Striatum:
Three-month-old homozygous Q175FDN HD mice were injected bilaterally into the striatum with formulation buffer (sham), low dose (5.2 X 109 gc/mouse) or high dose (1.3 X 1011 gc/mouse) AAV5-miHTT.

AAV5 distribution in the CNS:
*HD Transgenic mice received bilateral intra-striatal injections at 2 months of age with an AAV5 vector expressing green fluorescent protein (AAV5-GFP) and were harvested for immunohistochemistry at 1, 4 and 7 months post-injection. AAV5-GFP showed broad distribution at all time points evaluated and could clearly be observed in the striatum (site of injection), the hippocampus and deeper layers of the cortex (**Panel A**). To examine cellular tropism of AAV5 within the brain, HD mice received bilateral intra-striatal injections with a scramble miRNA tagged with GFP (AAV5-miScr-GFP). We qualitatively observed co-localization of GFP with NeuN (**Panel B**), DARPP-32 (**Panel C**) and GFAP (**Panel D**), demonstrating transduction of neurons, striatal medium spiny neurons and astrocytes, respectively. This shows broad cellular tropism, consistent with previous reports for the AAV5 serotype.*

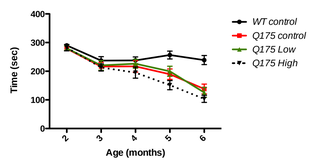
Experimental Design



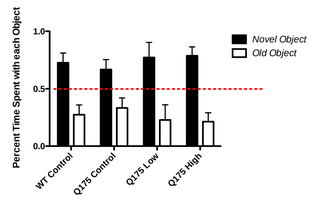
RESULTS

Behavioural Measures

A. Accelerating Rotarod

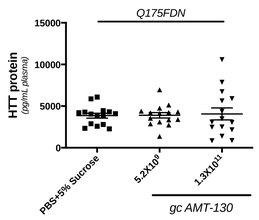


B. Novel Object Recognition

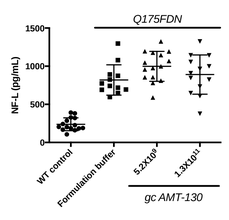


Plasma Biochemistry Measures

C. mHTT Protein



D. NF-L Protein



Brain Biochemistry Measures

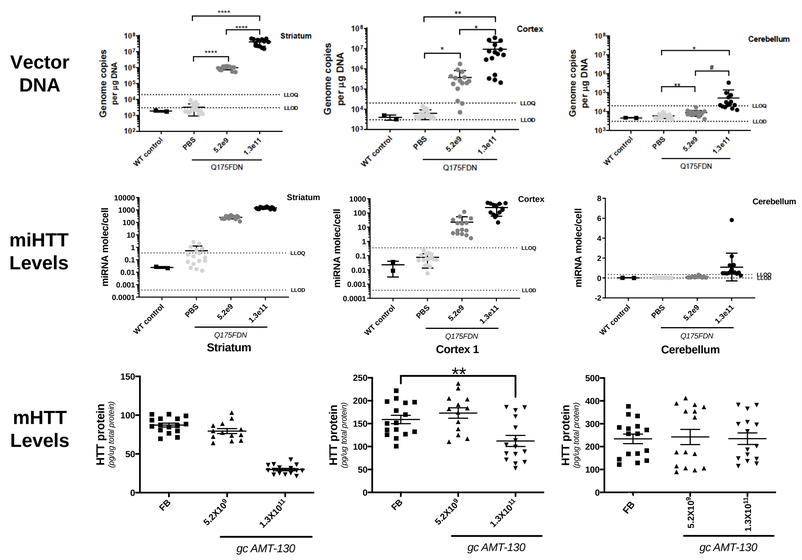
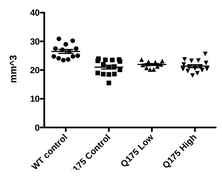


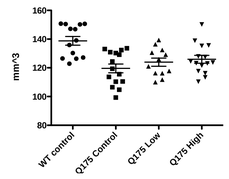
Figure 1. Behavioural, Plasma Biochemical, and Brain Biochemical measures in response to AAV5-miHTT therapy in Q175FDN mice.
Q175FDN mice have deficits in rotarod performance (A) but not novel object recognition (B) compared to wild-type mice. Neurofilament light chain protein (D) but not mHTT (C) was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

Structural Brain MRI Measures

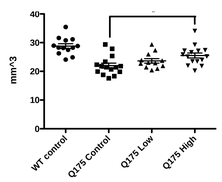
A. Striatal Volume



B. Cortical Volume



C. Hippocampal Volume



D. Thalamic Volume

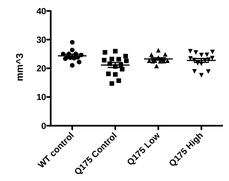


Figure 2. Structural MRI volume measures in response to AAV5-miHTT infusion in Q175FDN mice. *Compared to wild-type mice, Q175FDN mice had volume loss in the Striatum (A), Cortex (B), Hippocampus (C), and Thalamus (D). Intrastriatal infusion of AAV-miHTT did not significantly reverse this volume loss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

References:

Miniarikova J, Evers MM, Konstantinova P. (2018) Mol Ther. 4;26(4):947-962.

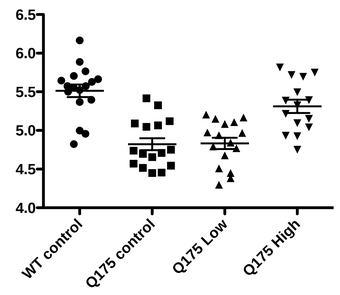
Miniarikova J, et al., (2016) Mol Ther Nucleic Acids. 22;5:e297.

Miniarikova J, et al., (2017) Gene Ther. 24(10):630-639.

Evers MM, et al., (2018) Mol Ther. pii: S1525-0016(18)30281-8.

Striatal MRS Measures

A. N-Acetyl Aspartate (tNAA)



B. Myo-Inositol (MI)

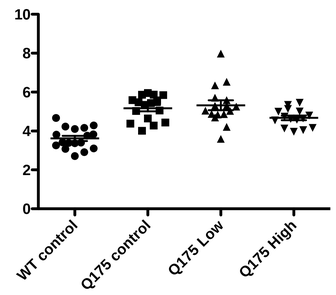


Figure 3. MRS changes in the striatum in response to AAV5-miHTT infusions in Q175FDN mice.
Q175FDN mice have decreased striatal levels of the neuronal marker tNAA and elevated levels of the glial marker MI similar to human HD. Treatment with AAV5-miHTT significantly reversed the changes in tNAA at the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

Correlation of mHTT with MRS Measures

A. tNAA vs. mHTT levels

B. MI vs. mHTT levels

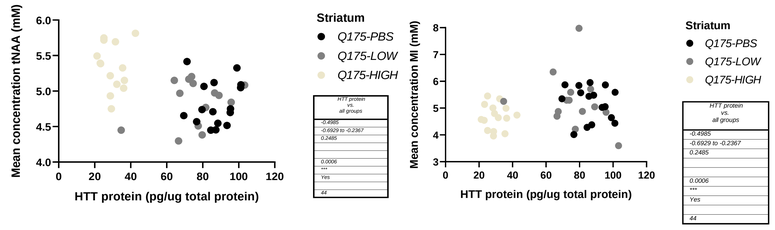


Figure 5. Correlation of striatal mutant huntingtin levels with tNAA and MI in Q175FDN mice following in AAV5-miHTT infusions.
There was a significant correlation between decreased levels of mHTT expression in the striatum of Q175FDN mice and higher levels of tNAA and lower levels of MI.

SUMMARY OF RESULTS

- **Intra-striatal administration of AAV5-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 AAV5-mihtt in Q175FDN mice.**
- **Q175 mice show robust regional volume lossin multiple brain regions at 6 months of age as measured by structural MRI.**
- **There was significant restoration of tNAA levels following 3 months of treatment with AAV5-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 AAV5-miHTT at 2-3 months of age lead to significant improvements in brain metabolites as measured by MRS.**
- **Silencing of mHTT in the brain continues to be a viable therapeutic strategy.**
- **Final point?**

Acknowledgements:

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