Sustained mutant huntingtin lowering in the brain and cerebrospinal fluid of Huntington disease minipigs mediated by AAV5-miHTT gene therapy

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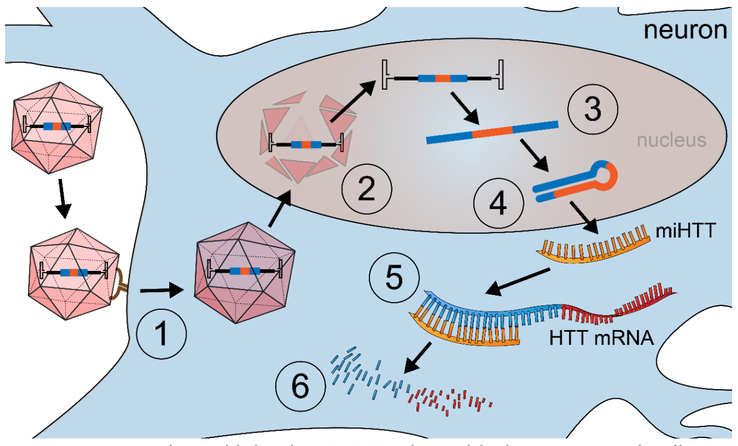
BACKGROUND

HTT-lowering therapies hold great promise to slow-down or halt neurodegeneration in Huntington disease (HD). We have developed an engineered microRNA targeting human huntingtin (HTT), delivered via adeno-associated viral vector serotype 5 (AAV5-miHTT), leading to efficient HTT-lowering in vitro and in vivo in rodent models. Transgenic HD (tgHD) minipigs, ubiquitously expressing human mutant HTT (548-amino acid N-terminal human fragment with 124 repeats), are suitable to study proof-of-mechanism of HTT-lowering in a large brain. In this ongoing study, we aim to assess the therapeutic window of our approach in a large animal model. We observed sustained target engagement and strong efficacy up to 1 year after one-time intrastriatal injection of AAV5-miHTT in tgHD minipigs.

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.*

OBJECTIVES

- **Surgical target acquisition:** evaluate feasibility of MRI-guided convection-enhanced delivery (CED) of AAV5-miHTT in tgHD minipigs
- **Biodistribution:** assess long-term distribution of vector DNA in different brain areas
- **Long-term efficacy:** measure miHTT and human HTT mRNA and protein expression in several brain regions
- **Biomarkers**: assess expression of mutant HTT protein in cerebrospinal fluid (CSF)

METHODS

Animals

- Young adult tgHD Göttingen minipigs (males and females, age 4-8 months), origin Libechov (n=30)
- Treatment groups:
  - Control (untreated)
  - AAV5-miHTT treated (1.2 x 10^{13} gc/animal)
- Per treatment group, interim sacrifices:
  - 6 months (n=3/group)
  - 12 months (n=4/group)
  - >24 months (n=8/group) *[still in in-life period]*

AAV5-miHTT administration

- MRI-guided CED (Renishaw pre-clinical drug delivery system), bilateral into caudate and putamen (100µL per structure) (maximum rate 3 µL/min) (**Fig. 2**).

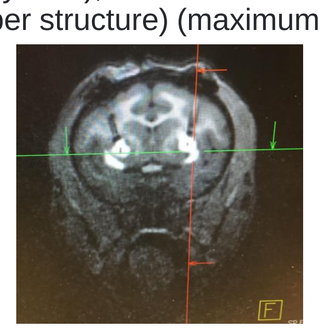
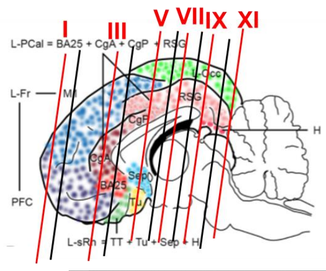


Fig 2. Representative MRI of the administration procedure. *Caudate and putamen filled structures were visualized with 2mM ProHance contrast agent.*

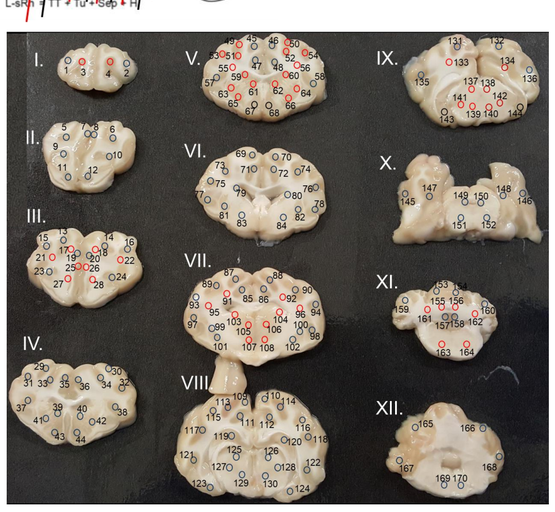
- CSF was collected pre- and post-injection (every 3 months)

- Brains were cut in 4mm slices, and 4mm punches taken:



6 months sacrifice:
n=27 punches/hemisphere

12 months sacrifice:
n=85 punches/hemisphere



- Brain punches were divided for bioanalysis:
  - DNA isolation → vector DNA (Q-PCR)
  - RNA isolation → miHTT (RT-QPCR)
  - Tissue lysate → mHTT protein (2B7-MW1 Singulex assay)

RESULTS

High and widespread vector DNA levels in the tgHD minipig brain 6-12 months after CED of AAV5-miHTT

- High vector transduction was observed throughout the brain of AAV5-miHTT injected animals, with peak levels in target regions (caudate, putamen), limbic areas (amygdala), cortical regions, and thalamus (**Fig. 4**).
- Vector DNA levels in the different brain regions were comparable at 6 and 12 months post-administration, indicating sustained AAV5-miHTT vector transduction.

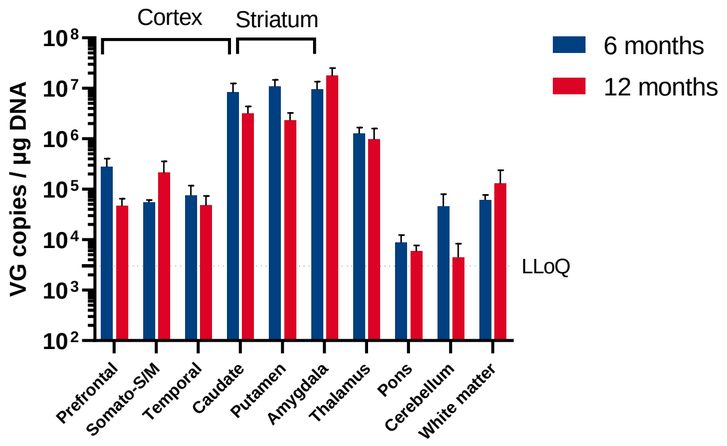


Fig. 4. Comparison of vector genome (VG) copies (gc/µg DNA) in tgHD minipig brain regions at 6 and 12 months post-AAV5-miHTT administration. *Bars represent average +/- SEM per brain region.*

- The extensive punching scheme throughout the whole cerebral cortex at 12 months post-injection, revealed a widespread vector DNA distribution, with detectable levels in all cortical regions analyzed (**Fig. 5**).

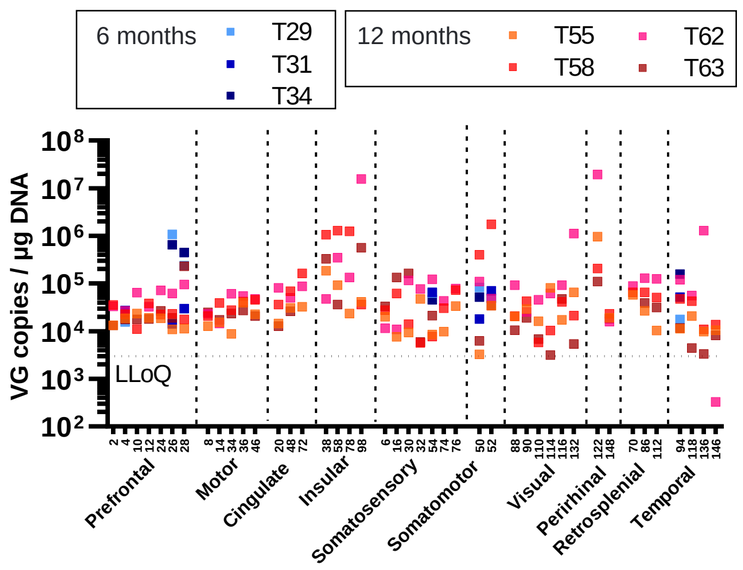


Fig. 5. Vector genome (VG) copies (gc/µg DNA) in tgHD minipig cortical areas at 6 and 12 months post-AAV5-miHTT administration. *Values are indicated per animal (6 months post-injection T29, T31, T34; 12 months post-injection T55, T58, T62, T63) and punch number / region.*

High and widespread miHTT expression across the brain 12 months after AAV5-miHTT administration

- Mature miHTT molecules were detected in virtually all brain areas from animals treated with AAV5-miHTT, in accordance to vector DNA levels (**Fig. 6**)

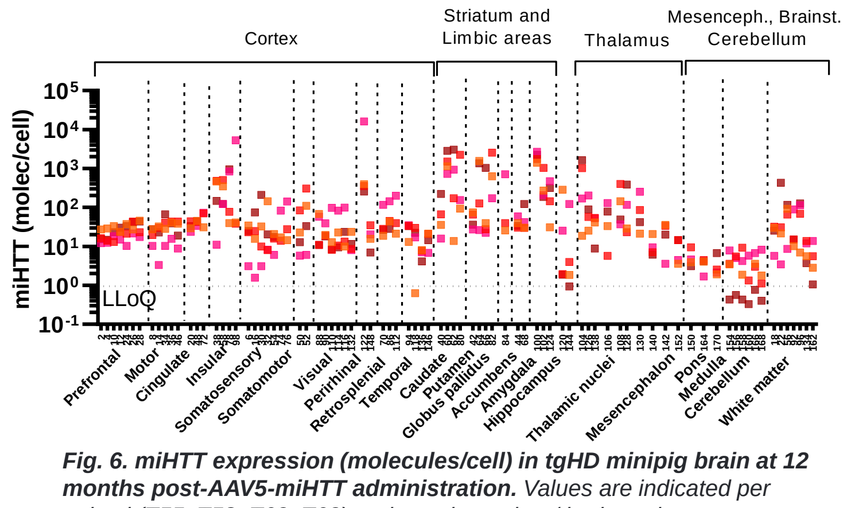


Fig. 6. miHTT expression (molecules/cell) in tgHD minipig brain at 12 months post-AAV5-miHTT administration. *Values are indicated per animal (T55, T58, T62, T63) and punch number / brain region.*

Mutant HTT protein lowering in brain tissue and CSF

- A strong and long-lasting mutant HTT protein lowering was observed across the brain (**Fig. 7A**), to a comparable extent at 6 and 12 months after AAV5-miHTT CED (**Fig. 7B**).

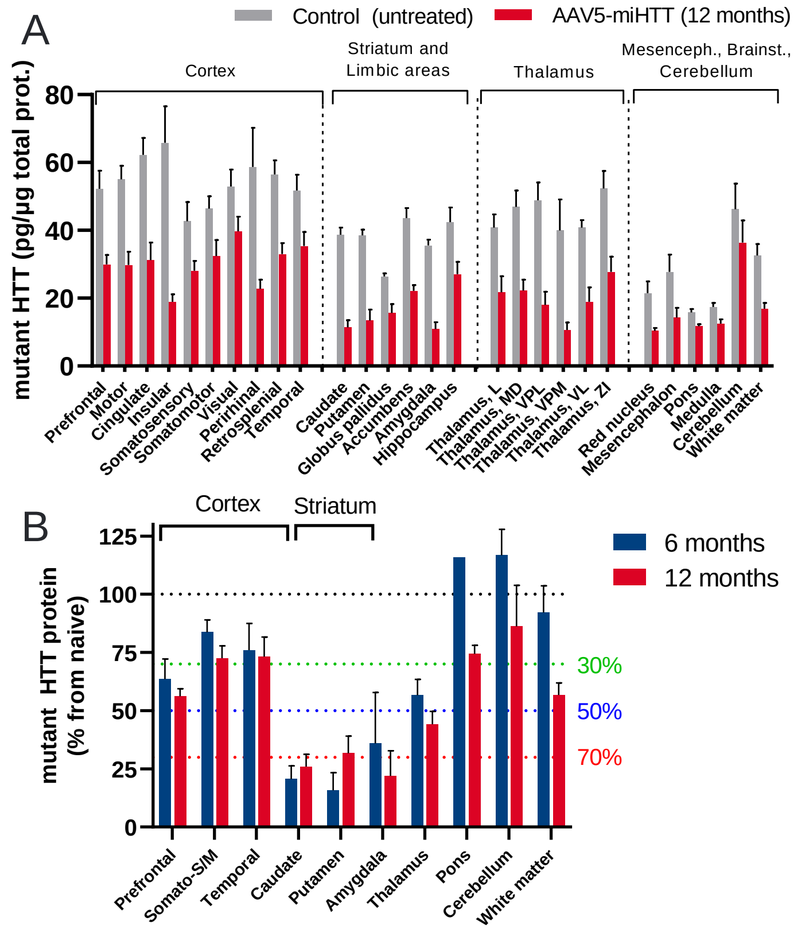


Fig. 7. Human mutant HTT protein in tgHD minipigs. (A) *mHTT protein (pg/µg total protein) in Control (naïve) and AAV5-miHTT treated animals (12 months post-injection).* ***(B)*** *Comparison of mutant HTT protein expression (% from naïve) at 6 and 12 months post-AAV5-miHTT administration. Bars represent average +/- SEM.*

- Mutant HTT protein levels in longitudinal CSF samples were analyzed up to 6 months post-injection. A strong reduction of CSF mutant HTT protein was observed from 3 months after AAV5-miHTT CED, compared to pre-dose (**Fig. 8**).

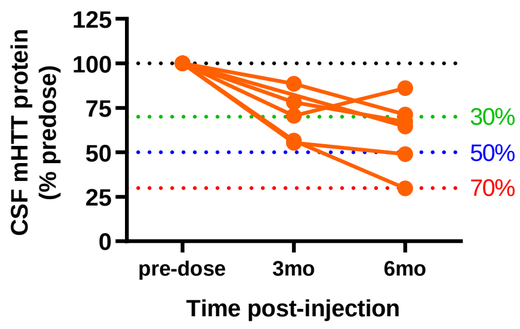


Fig. 8. Human mutant HTT protein expression in CSF. *Data are expressed as % from pre-dose levels (n=6 AAV5-miHTT injected animals, age >6 months at treatment).*

CONCLUSION

Here, we show strong, widespread and sustained (up to 12 months) target engagement and efficacy of one-time intrastriatal administration of AAV5-miHTT in tgHD minipigs. The current results of this ongoing study, on mutant HTT protein lowering in several brain regions, support the continuation of our program into the clinic.

ACKNOWLEDGMENTS

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