Assessment of the Novel AAV-Based miQURE™ Gene Therapy in SCA3 Animal Models

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BACKGROUND

Spinocerebellar ataxia type 3 (SCA3) is a progressive and fatal neurodegenerative disorder. Clinical manifestations include progressive gait ataxia with the involvement of cranial nerves. An expansion of a CAG trinucleotide repeat in the ATXN3 gene causes the accumulation of aberrant, toxic ataxin-3 protein in brain regions located in the posterior fossa. An ataxin-3 silencing approach was investigated using the next-generation miQURE™ technology (Miniarikova J, et al., 2016). Artificial microRNAs were engineered to downregulate the ATXN3 gene and packaged into adeno-associated viral vectors (AAV-miATXN3).

AAV5-miATXN3 and the miQURE™ technology

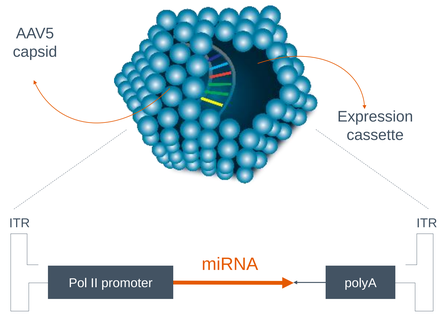

Fig. 1. Design and features of the miQURE™ technology

- *AAV-miATXN3 binds to cell-surface receptors and is internalized in the target cell (neurons and astrocytes)*
- *Transport to the nucleus and uncoating of the miATXN3 transgene occurs*
- *The hairpin structured precursor is transported to the cytoplasm and further processed to mature guide miATXN3*
- *No miRNA passenger strand is formed, strongly limiting the risk of off-target activity*
- *Mature miATXN3 is loaded in the RNA-induced silencing complex and binds ATXN3 mRNA, leading to degradation of the target transcript and protein knockdown*

STUDY AIMS

- Establish ataxin-3 knockdown potency of AAV-miATXN3 treatment in SCA3 mouse models
- Determine optimal route of administration to target the key affected SCA3 brain regions *in vivo*

METHODS

Design and *in vitro* testing of AAV-miATXN3

- miATXN3 candidates were designed and tested in luciferase assays (Martier R, et al., 2019)
- AAV-miATXN3 were found to be effective in iPSc neurons

AAV-miATXN3 injection in SCA3 knockin mice

- The F512 SCA3 knockin mouse model was generated by the Hübener-Schmid lab (Haas *et al,* 2020)
- Three miATXN3 candidates were injected in cisterna magna
- Ataxin-3 knockdown efficiency was determined by TR-FRET
- A lentiviral model expressing human ATXN3 cDNA was generated by the Pereira de Almeida lab (Gonçalves N, 2013)
- Lentivirus (atx3-72Q) was co-injected with AAV-miATXN3 bilaterally in striatum of wildtype mice

Test model	CAG repeat	Expression construct	Expression site
F512 knockin mouse[3]	304	Knockin, murine locus	Ubiquitous
SCA3-LV mouse[4]	72	Human cDNA	Striatum

Table 1. Mouse models used to establish AAV-miATXN3 efficacy

RESULTS

Cisterna magna injection results in efficient AAV uptake and miATXN3 expression in brainstem and cerebellum

- Several routes of AAV delivery were examined to target SCA3 relevant brain regions in mice (Martier R, et al., 2019)
- Cisterna magna injection was found to result in most efficient spread **(Fig 2)**

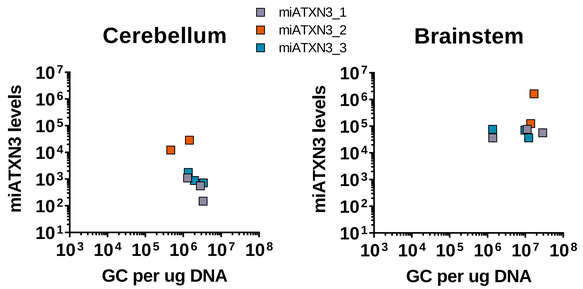

Fig. 2. Vector genome copies versus miATXN3 expression after cisterna magna delivery of AAV-miATXN3 in SCA3 knockin mice. *miATXN3 expression relative to formulation buffer, using U6 small RNA as reference gene and vector genome copies (GC).*

Mutant ataxin-3 is downregulated upon AAV-miATXN3 treatment, showing efficient target engagement

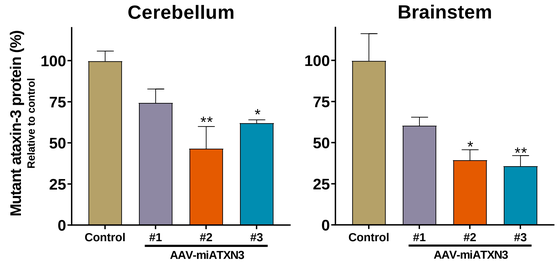

Fig. 3 Reduction of mutant ataxin-3 protein in the cerebellum and brainstem after cisterna magna delivery of AAV-miATXN3. *TR-FRET immunoassay was performed on tissue lysates to specifically detect the mutant ataxin-3 (no detection of wildtype mouse ataxin-3).*

AAV-miATXN3 prevents onset of ataxin-3 aggregates and neuronal dysfunction *in vivo*

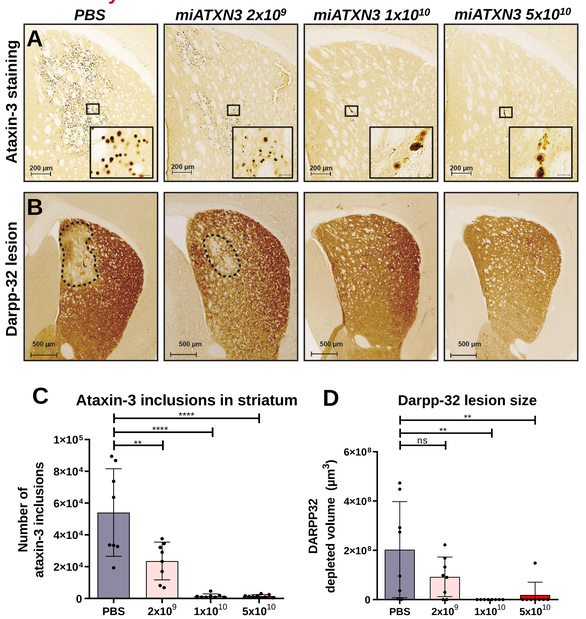

Fig. 4. Reduction of mutant ataxin-3 aggregates and restoration of dapp-32 expression in striatum. A) *Lentiviral expression of mutant ataxin-3 (72Q) results in ataxin-3 aggregates in striatum of mice.* ***B)*** *Mutant ataxin-3 leads to neuronal dysfunction (darpp-32 depleted area). Striatal injection with AAV-miATXN3 strongly improves the SCA3 phenotype, as exemplified by reduction in ataxin-3 inclusion number (quantified in* ***C****) and restoration of darpp-32 expression (quantified in* ***D****).*

Strong reduction of mutant ataxin-3 in SCA3 mouse models represents proof of mechanism of AAV-miATXN3 gene therapy

- Robust ataxin-3 protein lowering in the cerebellum and brainstem was seen in SCA3 knockin mice after AAV-miATXN3 injection in cisterna magna (**Fig 3**)
- Strong dose dependent ataxin-3 knockdown of over 90% was observed at RNA and protein level **(Fig 5)** in LV-SCA3 mice
- Ataxin-3 aggregates and neuronal dysfunction was completely rescued due to ataxin-3 knockdown **(Fig 4)**

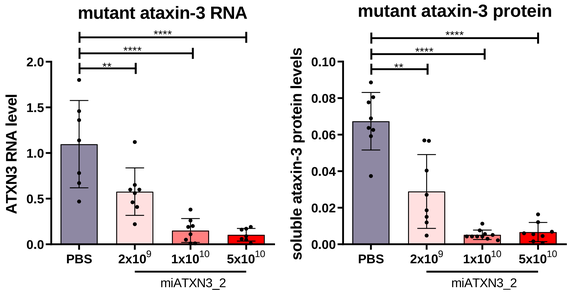

Fig. 5. Strong reduction in mutant ataxin-3 RNA and protein in striatum of LV-SCA3 mouse. *Three dosages of miATXN3_2 were injected in striatum together with a lentiviral construct expressing human ataxin-3. A robust dose dependent knockdown of mutant ataxin-3 RNA (left graph) and protein (right graph) was seen in striatum.*

CONCLUSIONS

An AAV delivered miRNA targeting ataxin-3 was designed and tested for efficacy in two mouse models of SCA3.

- Strong ataxin-3 knockdown at RNA and protein level was seen in both SCA3 mouse models
- Striatal injection alleviated all molecular SCA3 hallmarks in the LV-SCA3 mouse
- Cisterna magna injection resulted in robust ataxin-3 knockdown in cerebellum and brainstem of SCA3 knockin mice

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