AAV Gene Therapy in Juvenile Mice of STXBP1 Haploinsufficiency

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Poster 1.048

Background

- Syntaxin-binding-protein-1 (STXBP1) haploinsufficiency is a severe developmental epileptic encephalopathy (DEE) characterized by a loss of function of the STXBP1 and is associated with severe neuronal development delay, intellectual disability, and seizures. STXBP1 is an early-onset disease, and rapid treatment is needed after diagnosis to impact the disease progression. There are currently no efficacious treatments for the disease, and we explored the potential of a gene therapy (GT) to modulate STXBP1 haploinsufficiency in a STXBP1 knock-out (KO) mouse model.
- Two major splice variants are reported for STXBP1, including a long and a short isoform, and functional *in vivo* studies suggested that the long isoform would provide the most beneficial impact on disease symptoms (*see poster 3.051*).

Objective

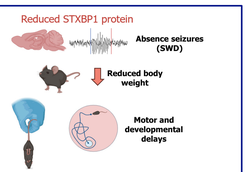
- In this study, we evaluated the efficacy and safety of a GT administered intracerebrally in the striatum of STXBP1 KO mice at juvenile age and measured the impact on multiple disease symptoms.

Methods

- STXBP1 heterozygous (HET) KO mice have been licensed from the University of Amsterdam.[1] Adeno-associated virus (AAV) constructs have been produced at UCB and encode the human long splice variant of STXBP1 under the control of a neuronal promoter. Animals have been injected at post-natal day (PND) 24-28 using a bilateral intra-striatal administration with either vehicle or AAV treatment (low dose of 2.6 E10 and high dose of 1.3 E11 vector genome copies [vgc]). Electroencephalogram (EEG) electrodes were implanted at the prefrontal cortex and cerebellum levels in parallel to the AAV injection, and the spike wave discharges (SWD) were recorded in group housing video-EEG wireless telemetry platform (24 hours/day) for several weeks. A battery of behavioral tests was performed to evaluate motor and cognitive performances. Analysis of transgene expression was performed in isocortex tissue using quantitative polymerase chain reaction (qPCR), Western blot, and immunohistochemistry.

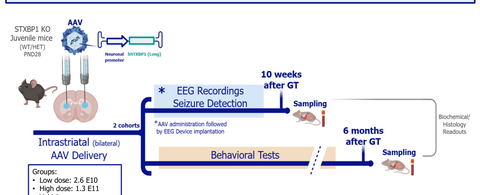
STXBP1 preclinical readouts

STXBP1 HET KO mice have reduced STXBP1 protein levels and present a robust disease phenotype with reduced body weight, frequent absence seizures, and motor and cognitive deficits*



*Compared with wild-type (WT).

Efficacy study protocol



BioRender-created figure. h, human.

Overview

QUESTION

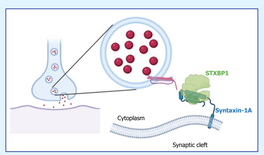
Does intracerebral delivery of a gene therapy at a post-symptomatic stage in juvenile mice provide therapeutic benefits?

INVESTIGATION

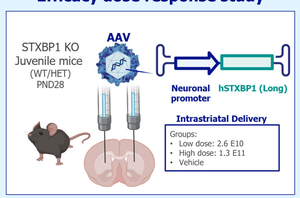
We used an adeno-associated virus vector to overexpress syntaxin-binding-protein-1 (STXBP1) human long variant in key disease brain areas using intra-striatal administration. Viral therapy was administered at post-natal day 28 at two different doses at a post-symptomatic stage. The impact of gene therapy was monitored up to 6 months using biochemical, electrographic, clinical, and behavioral readouts.

RESULTS

STXBP1 function



Efficacy dose response study



Symptoms	*Test*	Dose response: STXBP1 KO HET (PND28) Intra-striatal 2.6 E10	Dose response: STXBP1 KO HET (PND28) Intra-striatal 1.3 E11
Growth deficit	*Body weight (long term)*	No rescue	Not tested
Epilepsy	*EEG (SWD)*	Reduced	Reduced
	EEG (convulsive seizures)	Not observed	Detected
Motor dysfunction	*Hind limb clasping (dystonia)* *Wire hanging* *Open field*	No rescue	Not tested
Cognitive/developmental deficits	*Fear conditioning (associative learning memory)* *Nest building*	No rescue	Not tested

AAV, adeno-associated virus; EEG, electroencephalogram; h, human; HET, heterozygous; KO, knock-out; PND1, post-natal day 1; STXBP1, syntaxin-binding-protein-1; SWD, spike wave discharge; WT, wild-type.

CONCLUSIONS

Gene therapy with STXBP1 at juvenile post-symptomatic stage and administered intracerebrally (striatum) shows a strong STXBP1 protein overexpression in key brain areas. High-dose administration rapidly suppresses spike wave discharges but induces generalized seizures, whose mechanism remains unclear, and continuous electroencephalogram revealed short-term convulsive clusters. In contrast, a low dose is well tolerated and reduces spike wave discharges but does not confer an impact on broader disease symptoms.

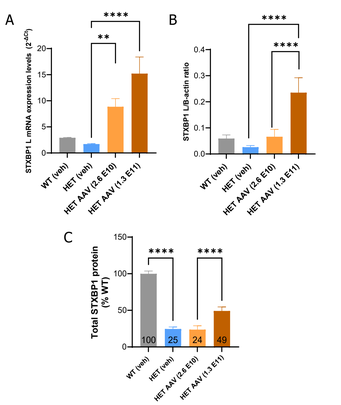
For a copy of the poster, scan:

Results

Distribution of STXBP1 protein following intra-striatal administration in juvenile HET KO mice

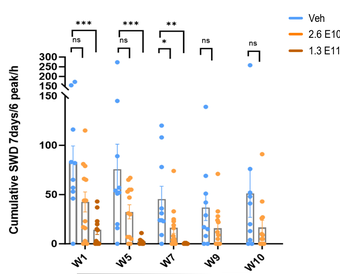
Intrastriatal administration at PND24-28 provides expression of STXBP1 in key brain areas such as frontal and motor cortex, striatum, and thalamus. hN, hemagglutinin.

Dose-dependent mRNA and protein increase of hSTXBP1 long variant in mouse isocortex



*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001. Transgene messenger RNA (mRNA) expression (A) was confirmed by qPCR in the isocortex of all translated mice with the two doses. Analysis of protein levels by Western blot in the isocortex indicated a significant increase of the long variant hSTXBP1 levels (B), with both doses, although the total STXBP1 levels (C) were only significantly increased with the high dose. veh, vehicle.

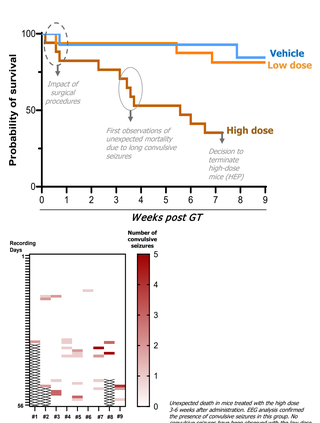
Rapid and sustained reduction of spike wave discharges (SWD) after high- and low-dose GT



SWD reduction %	2.6 E10	1.3 E11
W1	48%	82%
W5	57%	96%
W7	64%	99%
W9	58%	
W10	67%	

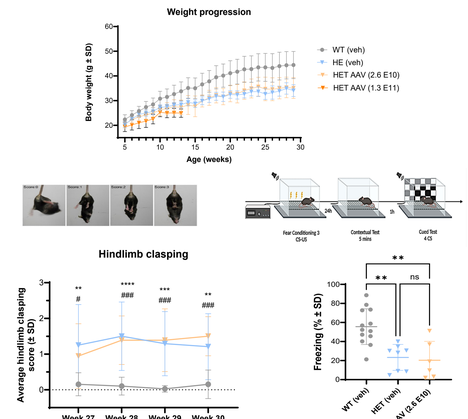
The high dose led to a rapid suppression of SWD already 1 week after GT injection and almost complete reduction of SWD after 5 weeks of treatment. The low dose translated into a reduction of SWD frequency of 60-70%, which was maintained until 10 weeks after treatment. ns, not significant; W, week.

Unexpected development of generalized convulsive seizures and mortality with the high-dose GT



Unexpected death in mice treated with the high dose 3-6 weeks after administration. EEG analysis confirmed the presence of convulsive seizures in this group. No convulsive seizures have been observed with the low dose. HEF, humane endpoint.

GT shows no effect on body weight progression, motor, or behavioral measures



*Left figure: **p <0.01; ***p <0.001; ****p <0.0001 (WT - veh vs HET - veh). #p<0.05; ###p<0.001 (WT - veh vs HET - AAV (2.6 E10)). Right figure: **p <0.01; ns, not significant. Animals treated with the low dose at PND28 were monitored for up to 6 months after GT. No changes were observed in body weight progression, motor symptoms (hindlimb clasping), or performance in the fear conditioning test (contextual and cued). SD, standard deviation.*

Conclusions

- Our study shows that the intracerebral (striatal) administration of an AAV GT in juvenile STXBP1 HET KO mice leads to expression of hSTXBP1 in key brain areas.
- We observed a rapid and dose-dependent reduction of SWD but have not been able to demonstrate impact on other disease symptoms.
- In our studies, the low dose was tolerated and safe up to 6 months. A high dose of AAV translates into convulsive seizures and unexpected mortality in this model.
- We conclude that intervention with an intracerebral GT at a post-symptomatic stage does not confer efficient impact on disease symptoms in STXBP1 HET KO mice.

References

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