AAV Mediated Overexpression of STXBP1 Variants in a Mouse Model of STXBP1 Haploinsufficiency

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Poster 3.051

Background

- Syntaxin-binding-protein-1 (STXBP1) disorders are linked to a loss of function of STXBP1 and are associated with severe neuronal development delay, intellectual disability, and seizures.[1] There are currently no efficacious treatments for the disease, and we explored the potential of a gene therapy (GT) approach to modulate STXBP1 haploinsufficiency in a relevant mouse model.
- Multiple splice variants have been reported for STXBP1, including a long and a short isoform generated by the splicing of the last exon.[2,3] Functional studies indicated that STXBP1 splice variants could play different roles in synaptic plasticity, and thus, may have an impact on disease conditions and therapeutic strategies.

Objective

- In this study, we evaluated the potential of a GT approach administered at neonatal age by overexpressing the long and short STXBP1 variants to modulate disease symptoms in a STXBP1 heterozygous (HET) knock-out (KO) mouse model.

Methods

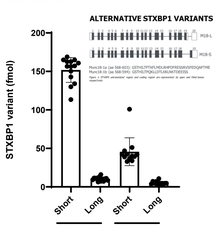
STXBP1 HET KO mice were licensed from the University of Amsterdam.[4] Adeno-associated virus (AAV) constructs encode the wild-type (WT) human STXBP1 and hemagglutinin (HA) tag under the control of a neuronal promoter. Animals were injected at post-natal day 1 (PND1) using intracerebroventricular (ICV) administration with either vehicle or AAV treatment. Electroencephalogram (EEG) recordings were performed in a group housing video-EEG wireless telemetry platform (24 hours/day). A battery of behavioral tests was performed to evaluate motor and cognitive performances. Biochemical analysis of transgene expression was performed in prefrontal cortex tissue using quantitative polymerase chain reaction (qPCR), Western blot, and liquid chromatography-mass spectrometry (LC-MS). Immunohistochemistry analysis using HA-tag antibodies was performed on fixed sagittal brain slices.

Results

STXBP1 SHORT VARIANT IS THE MOST PROMINENT ISOFORM EXPRESSED IN THE MOUSE BRAIN

The longer splice version shows a difference in the last 25 C-terminal amino acids and is reported to be expressed to a major part at the synaptic level and in gamma-aminobutyric acid (GABA)ergic neurons in the rat brain. The smaller splice version was localized in different cellular compartments and is more ubiquitously expressed in GABAergic and glutamatergic neurons.

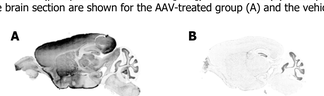
This figure shows quantitative LC-MS analysis of endogenous STXBP1 variants in the prefrontal cortex of WT and HET STXBP1 mice. The STXBP1 short variant is much more abundant than the long variant, and a significant decrease of both variants is observed in the STXBP1 HET mice. The ratio of long/short variant is increased in the caudal brain regions, and species differences in STXBP1 variant distribution have been reported earlier.[5]



HET, heterozygous; STXBP1, syntaxin-binding-protein-1; WT, wild-type.

GENE THERAPY (PND1, ICV) LEADS TO A WIDE BRAIN DISTRIBUTION OF THE STXBP1 TRANSGENE

We evaluated the brain distribution of HA-tagged STXBP1 expression following AAV treatment in mouse brain by immunohistochemistry (7 weeks post injection). HA tag staining was performed on saggital sections from HET mice injected with the HA-tagged STXBP1 long variant (A) and compared with vehicle (phosphate buffered saline [PBS])-treated mice (B). Examples of representative brain section are shown for the AAV-treated group (A) and the vehicle-treated group (B).



Overview

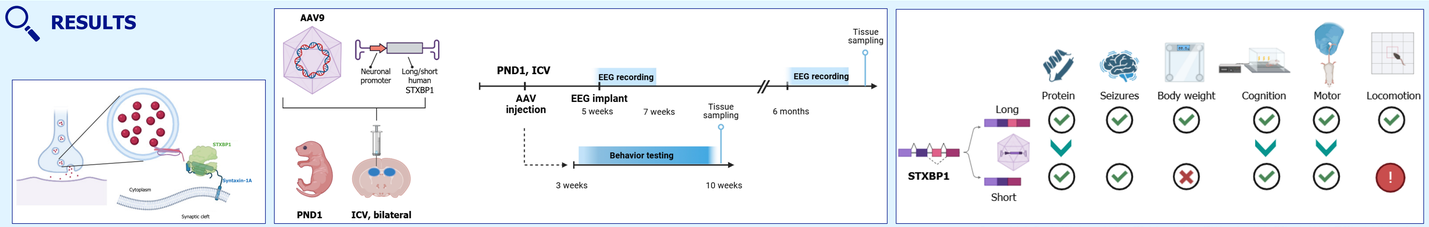
QUESTION

Can disease symptoms in a mouse model of syntaxin-binding-protein-1 (STXBP1) haploinsufficiency be rescued by a gene therapy administered at neonatal age?

INVESTIGATION

We used an adeno-associated virus vector to overexpress STXBP1 variants in large brain areas using intracerebroventricular (ICV) administration. Therapy was administered at post-natal day 1 (PND1) at a pre-symptomatic stage to achieve maximal transgene distribution and therapeutic efficacy. The impact of gene therapy was monitored up to 6 months using biochemical and behavioral readouts.

RESULTS



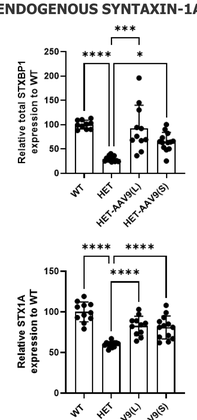
Figures created using Biorender. AAV, adeno-associated virus; EEG, electroencephalogram; ICV, intracerebroventricular; PND1, post-natal day 1; STXBP1, syntaxin-binding-protein-1.

CONCLUSIONS

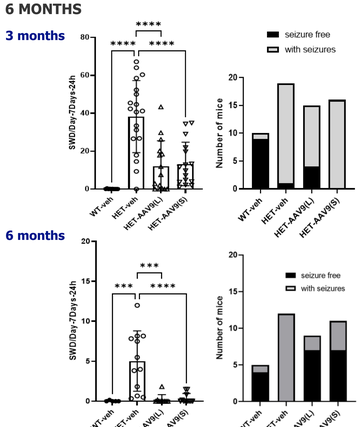
Gene therapy with STXBP1 at PND1 and administered ICV demonstrated a strong impact on STXBP1 protein expression in the brain and on disease symptoms in the heterozygous mouse model. The overexpression of the long STXBP1 variant showed the most robust and consistent effects across all disease symptoms, whereas the short variant showed a smaller impact and demonstrated a negative effect on locomotor activity.

For a copy of the poster, scan:

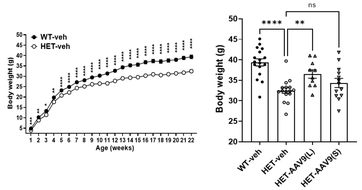
GENE THERAPY RESCUES STXBP1 PROTEIN EXPRESSION AND ENDOGENOUS SYNTAXIN-1A LEVELS



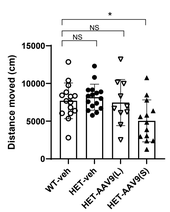
GENE THERAPY WITH STXBP1 PRODUCES ROBUST REDUCTION OF SPIKE WAVE DISCHARGES UP TO 6 MONTHS



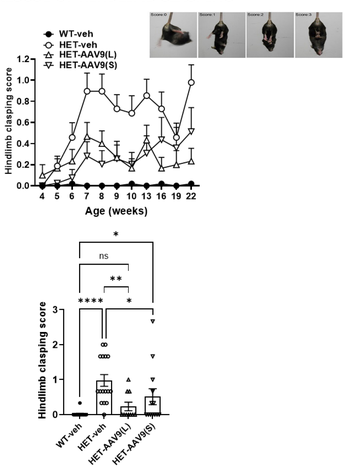
GENE THERAPY WITH STXBP1 LONG VARIANT IS ABLE TO RESCUE BODY WEIGHT



GENE THERAPY WITH STXBP1 SHORT VARIANT PRODUCES A DECREASE IN GROSS LOCOMOTOR ACTIVITY IN THE OPEN FIELD TEST

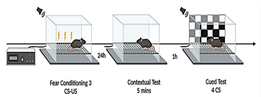


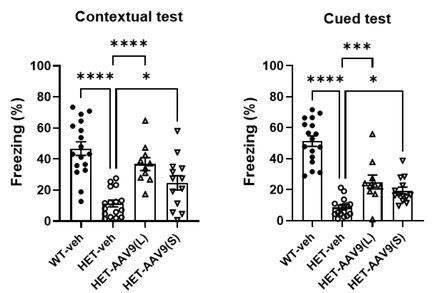
GENE THERAPY IMPACT ON MOTOR SYMPTOMS: RESCUE OF HINDLIMB CLASPING



*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. AAV, adeno-associated virus; h, hours; HET, heterozygous; (L), long; ns/NS, not significant; (S), short; STX1A, syntaxin-1A; STXBP1, syntaxin-binding-protein-1; SWD, spike wave discharge; veh, vehicle; WT, wild-type.

GENE THERAPY IMPROVES COGNITIVE BEHAVIOR IN THE FEAR CONDITIONING TEST





*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. AAV, adeno-associated virus; CS, conditioned stimulus; h, hour(s); HET, heterozygous; (L), long; min, minute; (S), short; US, unconditioned stimulus; veh, vehicle; WT, wild-type.

Conclusions

- Administration of a gene therapy at neonatal age by ICV translates into a large brain distribution of the STXBP1 transgene.
- AAV delivery of STXBP1 is able to rescue haploinsufficiency of STXBP1 protein levels and increases endogenous levels of syntaxin-1A.
- STXBP1 overexpression has a robust impact on seizure activity, achieving a high level of seizure freedom, which is maintained up to 6 months.
- The overexpression of the STXBP1 long isoform has the strongest impact on disease symptoms, including the rescue of body weight, motor symptoms, and cognitive deficits.
- The delivery of the STXBP1 short isoform has no effect on body weight, and we identified a negative impact on locomotor activity.
- We suggest that the delivery of the STXBP1 long isoform may offer a relevant option to develop a gene therapy in STXBP1 disorders.

References

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3. Ramos-Miguel A, et al. *Mol Neurodegener* 2015;10:65.
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