THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

CRISPR/Cas9 Genome Modifications to *SLC6A1b* in *Danio Rerio*

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Introduction

- *Solute Carrier Family 6 member 1* is a rare neurodevelopmental disorder (*SLC6A1-NDD*) affecting children
- The mutation in the *SLC6A1* gene impacts the gamma-aminobutyric acid (GABA) transporter protein type 1 (GAT1) resulting in the inability to efficiently remove GABA from the synaptic cleft and affecting the reuptake that subsequently leaves a surplus of GABA in the cleft
- The clinical presentation is a wide spectrum including epilepsy, mild to severe intellectual disorders, behavioral disorders, and mild to severe Autism Spectrum Disorder (ASD)
- *SLC6A1* variants are normally de-novo missense mutations within conserved regions resulting in a loss-of-function (LOF) of GAT-1

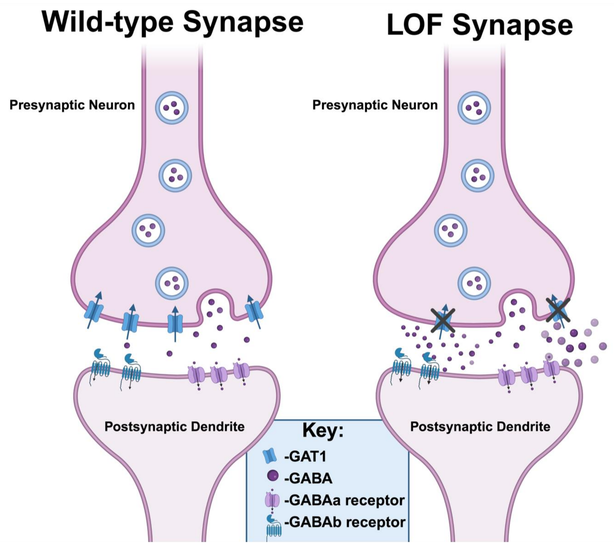

Figure 1. Healthy Synaptic Function using GAT-1 vs. Synaptic Function with Loss of Function of GAT-1. GAT-1 works to reuptake GABA from the synaptic cleft back into the presynaptic terminus. In *SLC6a1* mutations, the loss of function of the GAT-1 protein causes an increase of extracellular GABA in the synaptic cleft leading to over-excitation of the post-synaptic neuron.

References

1. Johannesen, K. M., Nielsen, J., Sabers, A., Isidor, B., Kattentidt-Mouravieva, A. A., Zieglgänsberger, D., Heidlebaugh, A. R., et al. (2023). The phenotypic presentation of adult individuals with SLC6A1-related neurodevelopmental disorders. *Frontiers in Neuroscience*, *17*, 1216653.
2. Lindquist, B. E., Voskobiynyk, Y., Goodspeed, K., & Paz, J. T. (2023). Patient-derived SLC6A1 variant S295L results in an epileptic phenotype similar to haploinsufficient mice. *Epilepsia*, *64*(10), e214–e221.
3. Lindquist, B. E., Voskobiynyk, Y., & Paz, J. T. (2021). Electrobehavioral phenotype and seizure pharmacosensitivity in a novel mouse model of patient-derived *SLC6A1* S295L mutation-associated neurodevelopmental epilepsy. *BioRxiv*.
4. Images created with BioRender.com

Methods

- Primers were chosen for use based on length, GC content, melting point, and amplicon size according to Benchling and ChopChop
- Chop Guides 1 and 5 were chosen for injection due to respective on target scores of 74.83 and 64.97

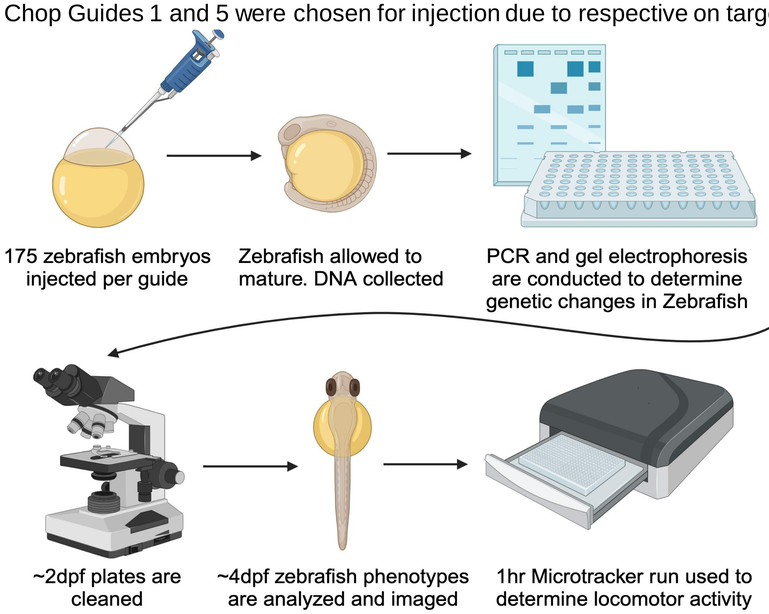

Figure 2. Depiction of the research methodologies. Two CRISPR/Cas9 guides were chosen based on Benchling scores and injected into zebrafish. PCR and gel electrophoresis assessed their DNA-cutting effectiveness. After 36 hours, dead embryos were removed, and live ones observed. At 80 hours, remaining fish were phenotypically analyzed, and a microtracker assay compared locomotion. Five embryos per guide underwent DNA sequencing to detect changes.

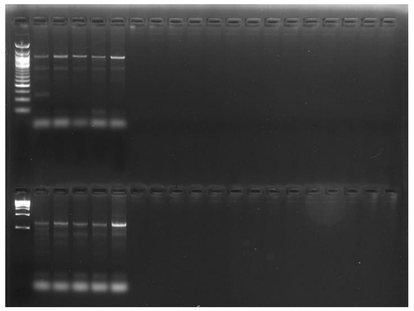

Figure 3. Gel Electrophoresis Results. To gauge cutting efficiency, gel electrophoresis was conducted, and interesting bands were identified. A master mix was made with both guide 5 (top run) and guide 1 (bottom run). A 50bp DNA ladder was used for chop 5 and a 1kb bp ladder was used for chop 1. The gel ran at 180V for 45 minutes.

Conclusion & Future Directions

- Embryos subjected to CRISPR Cas9 exhibited significant phenotypic variations including bent tails, shortened tails, heart edemas, mortality, and other developmental deformities.
- Further analysis of embryos using a MicroTracker assay to measure total locomotive activity revealed that chop 5 demonstrated increased significance compared to chop 1.
- Chop 5 is predicted to induce a greater loss of function by causing early truncation of the protein responsible for GABA reuptake.
- Inject more embryos with chop guide 5 while utilizing imaging, phenotyping, and locomotive assays to establish validity
- Allow zebrafish to mature longer to perform additional assays and classify phenotypes into subcategories

Results

	Bent Tails	In Chorion	Seizures	Other Abnormalities	Dead
Chop 1 Guide	7%	3%	0.6%	shortened tail-0.6% heart edema-0.6%	18%
Chop 5 Guide	10%	5%	1%	no cephalic development-0.6%	36%

Figure 3. Visual Phenotypic Results. Variations in phenotypes were observed at approximately 4dpf. Among the variations were a high number of bent tails, developmentally delayed fish (still in chorion), seizure activity, etc. Chop 5 has shown to have more significant results with higher rates of phenotypic abnormalities.

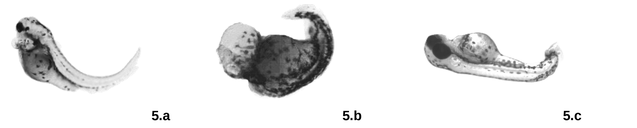

Figure 5. Captured microscope images of CHOP 5 zebrafish phenotype 72 hours after guide injection. 5.a presents with a moderately bent tail. 5.b presents with no face and severe deformity. 5.c presents with a severely bent tail.

Figure 4. Protein Blast of WT protein sequence vs. protein sequence of Chop 5 Injected Embryo. A4 denotes an interesting sample from the group of zebrafish injected with the chop 5 guide. The protein blast shows various substitutions of amino acids as well as an early truncation of the A4 protein at amino acid 43.

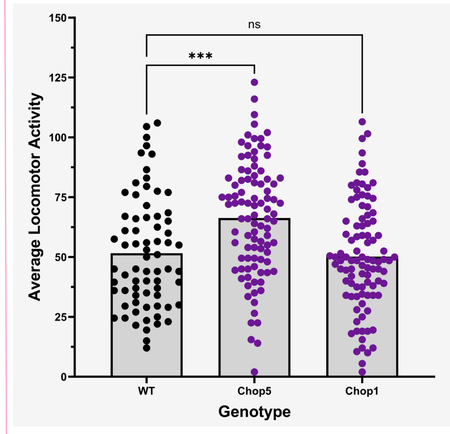

Figure 6. Microtracking data of wildtype (WT) vs. CHOP 1 and CHOP 5 guides. A three-star significance was found in the difference of movement between CHOP 5 and WT movement with CHOP 1 presenting no significant change .