CRISPR/Cas9 Genome Modifications to SLC6A1b in Danio Rerio

Jackie Brunson1, Adrienne Cooley1, Ansley Montgomery1, Gabrielle Smith2,3 and Cameron M. Crowder, Ph.D.1,2,3

Undergraduate Neuroscience Program1, Hugh Kaul Precision Medicine Institute2, Department of Neurobiology3

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

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

Results

<table>
<thead>
<tr>
<th>Guide</th>
<th>Phenotypes</th>
<th>Seizures</th>
<th>Other Abnormalities</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chop 1</td>
<td>7%</td>
<td>3%</td>
<td>0.6%</td>
<td>18%</td>
</tr>
<tr>
<td>Chop 5</td>
<td>10%</td>
<td>5%</td>
<td>1%</td>
<td>36%</td>
</tr>
</tbody>
</table>

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. 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 4. Protein Blast of WT protein sequence vs. protein sequence of Chop 5 Injected Embryo. A4 denotes an interesting variation. 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 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 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.

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

References

4. Images created with BioRender.com