In the past 20 years, autism spectrum disorder prevalence has increased by 600%1. The neurodevelopmental disorder, characterized by impaired communication, impaired reciprocal social interaction, and restricted or repetitive patterns of behavior or interests, can be partially accounted for by rare mutations in multiple genes2. There is also strong evidence that epigenetic factors, like DNA methylation, play a role in the symptoms associated with autism. One gene associated with autism is SHANK3 which is involved in support of synapses and maturation of dendritic spines3. It is hypothesized that the SHANK3 methylation disrupts the signaling that occurs between neurons, yet, *it is unclear how methylation directly affects SHANK3 and synaptic function.*

My**primary goal**is to determine how methylation of SHANK3 affects synaptic function using zebrafish as a model.  Zebrafish are excellent models for autism due to their genetic similarity to humans, transparency, quick development, and clear mutant phenotype, abnormal response to touch4. My **hypothesis**is that selectively targeting and knocking out methylation sites of SHANK3 will restore synapse function. **My long term goal** is to find a treatment for hyper-methylation of SHANK3 in autism patients in order to improve cognitive outcomes.

**Aim 1: Determine conserved SHANK3 methylation sites necessary for synapse function.**

**Approach:** MethPrimer will be used to identify potential methylation sites on the SHANK3 gene zebrafish homolog obtained from Ensemble. I will then use CRISPR Cas-9 to selectively knock out the conserved amino acids in wild type and SHANK3 mutant zebrafish and screen for the abnormal response to touch phenotype characteristic of other SHANK3 mutants to determine if the regions play a role in synapse function. **Hypothesis:**I hypothesize that knocking out methylation sites will result in restoration of normal response to touch. **Rational:** This will allow me to determine if selectively targeting and knocking out methylation sites can restore normal synaptic function in mutant zebrafish.

**Aim 2: Determine how methylation affects synapse function in SHANK3 mutants.**

**Approach:** RNASeq will be used to determine what genes are differentially expressed in the brain of wild-type and SHANK3 methylation mutant zebrafish. The genes will then be sorted by Gene Ontology function, specifically sorting for genes involved in synaptic signaling and social behavior. I will then use CRISPR/Cas9 to create zebrafish knockouts of the identified genes and screen for the abnormal response to touch phenotype. **Hypothesis:**I hypothesize that SHANK3 methylation will result in differentially expressed genes involved in signaling and social behavior. **Rational:** How methylation of SHANK3 affects synaptic function is unclear. By analyzing the changes in RNA expression levels of synaptic genes, I will be able to determine what genes are affected by changes in methylation.

**Aim 3: Quantify SHANK3 interacting protein levels in wild type and mutant SHANK3 zebrafish.**

**Approach:** Using the SHANK3 wild type and mutants found in aim 2, I will quantify protein levels in each treatment group using ITRAQ. CRISPR/Cas 9 will be used to knock out SHANK3 methylation sites in mutant and wild type zebrafish. Protein levels will be quantified again using ITRAQ. I will also screen for abnormal response to touch to confirm the role in synaptic transmission. **Hypothesis:** I hypothesize that mutant zebrafish will have higher levels of protein expression following SHANK3 methylation site knockout. **Rational:** This will allow be to determine if methylation site knockout successfully restores protein interactor levels in SHANK3 mutant zebrafish.

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