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**Attention Deficit Hyperactivity Disorder and the LPHN3 gene: Specific Aims**

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neuropsychological disorders. It is broadly characterized by individuals who have difficulty paying attention, controlling impulse behavior, and hyperactivity (1). ADHD is known to have a strong genetic component with large scale twin studies showing heritability to be anywhere from 75-91% (2) and one gene associated is LPHN3. LPHN3, an adhesion G-protein coupled receptor (GPCR), is almost exclusively expressed in the brain and mutations leads to a loss in synaptic density (3). Common ADHD medications rescue behavioral phenotypes in zebrafish (4), however little is known about the molecular mechanisms LPHN3 regulates in mediating synaptic density and behavior.

My **primary goal** is to better understand the molecular mechanisms necessary for behavior regulated by LPHN3. I **hypothesize** that specific domains in LPHN3 are necessary to mediate proper synaptic density and behavior. My **long-term goal** is to discover a new way to treat ADHD patients with mutant LPHN3.

**Specific Aim #1**: **Identify protein domains necessary for regulating proper synaptic density and behavior.**

***Rationale***: Synaptic density is important for neuron communication and individuals with ADHD have less synaptic density. Discovering which domains in LPHN3 influence synaptic density will help understand how the molecular function of this G-protein plays in mediating behavior.

***Approach***: Using Clustal Omega, SMART, and PFAM I will identify conserved domains and amino acids between zebrafish and humans in LPHN3. Then, using CRISPR-Cas9, I will create constructs where each conserved domain is deleted and then I will create transgenic zebrafish lacking certain domains. Synaptic density will be measured in mutant zebrafish by immunoprecipitation of PSD-95 from brain tissue, and behavioral phenotype will be characterized by an increased locomotion activity.

***Hypothesis***: I predict that the loss of the hormone receptor domain will lead to decreased synaptic density and behavior abnormalities.

**Specific Aim #2**: **Identify small molecules that rescue synaptic density and behavior defects in LPHN3 mutants.**

***Rationale***: Small molecules are currently being used to treat ADHD patients. Identifying small molecules specific to LPHN3 could lead to new treatments.

***Approach***: I will use a focused chemical genetic screen, that targets GPCRs, in order to discover small molecules that rescue LPHN3 mutants which have decreased synaptic density and behavioral defects.

***Hypothesis***: Synaptic density and behavioral defects can be recovered by small molecules that target domains in the extracellular region such as the hormone receptor.

**Specific Aim #3**: **Identify and analyze novel protein-protein interactions in wild type, mutant, and small molecule treated mutant LPHN3 phenotypes.**

***Rationale***: Discovering protein interaction networks in wild type, mutant, and small molecule treated mutant zebrafish will provide insight as to how synaptic density and defective behavioral phenotypes are regulated in mutant LPHN3.

***Approach***: Using a TAP-tag MS analysis to identify interacting protein networks in wild type, mutant, and small molecule treated mutant zebrafish.

***Hypothesis***: With treatment of previously identified small molecules in Aim 2, I predict that there will be more protein interactions recovered with proteins involved with synaptic density.

References

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