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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. Null and mutant LPHN3 leads to a loss in brain volume (3). Common ADHD medications rescue behavioral phenotypes in zebrafish (4), however little is known about the molecular mechanisms LPHN3 regulates in brain development and behavior.

My **primary goal** is to better understand the molecular mechanisms necessary for brain development and behavior regulated by LPHN3. I **hypothesize** that specific domains in LPHN3 are necessary to mediate proper brain development and behavior. My **long-term goal** is to discover a new way to treat individuals with mutant LPHN3 in order for them to have proper brain development and not develop ADHD.

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

***Rationale***: Individuals with ADHD have smaller brains than unaffected individuals. Null zebrafish have decreased brain volume and are hyperactive. Discovering which domains in LPHN3 influence proper brain development and behavior will help understand how LPHN3 affects ADHD.

***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. Then I will create transgenic zebrafish lacking each domain. Brain volume will be measured in mutant zebrafish by in vivo MRI and hyperactivity will be observed by swimming patterns.

***Hypothesis***: I predict that the loss of extracellular domains will be most important in proper brain development.

**Specific Aim #2**: **Identify small molecules that assist in brain development and recover behavior defects in LPHN3 mutants.**

***Rationale***: Small molecules are currently being used to treat ADHD patients. Stimulants in particular increase synaptic activity in ADHD patients and help recover ADHD like behavior. Identifying small molecules specific to LPHN3 could lead to new treatments for LPHN3 mutants.

***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. I will measure brain volume and behavior as well at each lifecycle stage to see if and when brain development and behavior can be recovered.

***Hypothesis***: Brain development and behavioral defects can be recovered by small molecules that target domains in the extracellular region.

**Specific Aim #3**: **Identify protein-protein interaction responsible for proper brain development in LPHN3 mutants.**

***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 co-immunoprecipitation at each life cycle stage of mutant, mutant treated, and wild type zebrafish I will identify crucial protein interactions that are necessary for proper brain development and behavior. I will also identify if any small molecules are able to recover crucial protein interactions in 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 in brain development and behavior.

References

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