**X-Linked Adrenoleukodystrophy (ALD)** results from the breakdown of the myelin in the brain. Myelin is a protective layer that surrounds the axons, and loss of this layer leads to the inability to control muscles and brain function. One gene associated with ALD is ABCD1 which is responsible for the transport of long-chain fatty acids into the peroxisome where they are broken down through beta oxidation. The lack of ABCD1 can cause buildup of long-chain fatty acids, which are toxic for myelin. ALD primarily affects males, *but It is unclear why there are sex-specific brain defects on myelin.*

My **objective** is to determine how ABCD1 mediates the proper maintenance of myelin between the sexes. I **hypothesize** that loss of ABCD1 affects males more because of hormonal differences involved in the maintenance has protein interactions with genes involved in protection of myelin, and a mutation will cause these genes to be downregulated more in males. Zebrafish is an excellent model organism because it has a well-studied nervous system which amenable to genetics and cell biological assays, and the structure of myelin is well conserved and can be assayed The **long-term goal** of this study is to determine how myelin is properly maintained by the peroxisome-associated gene ABCD1 between male and females.

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**Aim 1**: Determine conserved sequences in ABCD1 that are important for Myelin maintenance between male and females

**Approach:** Using NCBI and Ensemble I will obtain the ABCD1 homologs and determine conserved amino acids in abc membrane 2 that are important for maintaining myelin function in males versus females. I will mutate specific residues in zebrafish and look for loss of myelin in males versus females.

**Hypothesis:** Amino acid S269A of ABCD1 may be important for sex-specific defects.

**Rationale:** Mutations to ABCD1 in S269 in abc membrane 2 should result in improper myelin maintenance in males.

**Aim 2:** Identify necessary genes necessary for myelin maintenancein males and females.

**Approach:** I will perform RNA-SEQ on Wild type and ABCD mutants from Aim 1 to determine which gene ontology groups are differentially expressed between male and females. Genes that influence myelin preservation will be looked at in detail.

**Hypothesis:** Genes involved in myelin maintenance and peroxisome function will be downregulated to a greater extent in males.

**Rationale:** The breakdown of myelin of is the primary phenotype seen in ALD patients, particularly males.

**Aim 3:** Identify male-specific proteinthat mediate peroxisome function of ABCD1 that are important for myelin preservation.

**Approach:** I will perform on iTRAQ on wildtype and ABCD1 mutants made in aim 1. Mass spec will be used to determine the differences of protein levels seen between each group.

**Hypothesis:** Male-specific proteins important for myelin maintenance will be identified by comparing Wild type and ABCD1 mutant males and females.

**Rationale:** Males have more severe ALD phenotypes, so protein levels necessary for the maintenance of myelin might be lower in males.

References:

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