**X-Linked Adrenoleukodystrophy (ALD)** results from the breakdown of the myelin in the brain. Myelin is the protective layer of the axon, and the lack of this can cause improper transfer of signals. This signal defect affects the ability to control muscles and think. The gene most associated with ALD is ABCD1. It produces the adrenoleukodystrophy protein (ALDP). ALDP is found in peroxisomal membrane and is responsible for the transport of long-chain fatty acids into the peroxisome where they can be broken down. The peroxisome’s main function is to break down very long-chain fatty acids (VLCFA) through beta oxidation. The lack of this protein can cause buildup of VLCFA. High levels of this can be toxic to the presence of myelin in the brain. ALD affects males but it can be seen in females as well. It is unknown *why ALD is seen to affect males more severely than females given the similar expression levels of ABCD1 in both.*

My **objective** is to determine how ALDP interacts with different genes in males and females. I **hypothesize** that differential expression in males and females could result in more severe cases in males than females. The model organism used for this study will be the Mus musculus because it is very will conserved for the gene ABCD and contains an X and Y chromosome. The **long-term goal** of this study is to use the knowledge of this study to determine treatment options for both males and females.

**Aim 1**: Determine conserved sequences in ABCD1 in Mus musculus.

**Approach:** Using NCBI and Ensemble we could look at the conservation of the ABCD1 AA sequence. ABCD1 can be mutagenized via RNAi or Crispr in zebrafish to look for proper mutation phenotypes. This will also give us an indication of how well the gene is conserved.

**Hypothesis:** Model organisms with high conservation of ABCD1 will show the same symptoms as they do in males and females in humans.

**Rationale:** It is important to know that the model organism the gene relatively conserved. The more conserved ABCD1 is, the better the results of the experiment will be.

**Aim 2:** Look at gene expression of differentially expressed genes in males and females of Mus musculus.

**Approach:** I will use CRISPR on zebrafish males and females to knock out the ABCD1 gene. The zebrafish will be observed for mutants as well as the severity of the phenotypes. Then RNA SEQ will be done on all the fish and the expression levels of the genes will be recorded.

**Hypothesis:** ABCD1 mutation in zebrafish will show differential expression between males and females. I suspect that there might certain hormone in either of the two to be affected more than the other.

**Rationale:** I can see whether certain genes in the protein network or other genes are being affected more in males than they are in females. Genes responsible in myelination could be big factor in why there are differences between the two.

**Aim 3:** Quantify protein levels of ABCD1 in Mus musculus mutants between males and females.

**Approach:** Following the same approach as Aim 2 except tagging the ABCD1 gene with GFP. Flow cytometry can quantify the levels of GFP to give us an idea of ABCD1 protein levels. Western blot will be used to detect the amount of protein as extra data.

**Hypothesis:** I believe that the translation of ABCD1 will be higher in zebrafish mutant females than in males. If other genes are not the problem, then it must have to do with the levels of ABCD1.

**Rationale:** The levels of protein is crucial to understanding why symptoms are worse males. If higher levels of ABCD1 protein is present in females, it could indicate that there is something allowing for more translation of ABCD1. More protein would then suggest the milder symptoms in females.

References:

[1]ABCD1. (n.d.). Retrieved from <https://www.sciencedirect.com/topics/medicine-and-dentistry/abcd1>

[2]Berger, J., Pujol, A., Aubourg, P., & Forss-Petter, S. (2010, July). Current and future pharmacological treatment strategies in X-linked adrenoleukodystrophy. Retrieved March 3, 2020, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2967711/>

[3]Strachan, L. R., Stevenson, T. J., Freshner, B., Keefe, M. D., Miranda Bowles, D., & Bonkowsky, J. L. (2017, September 15). A zebrafish model of X-linked adrenoleukodystrophy recapitulates key disease features and demonstrates a developmental requirement for abcd1 in oligodendrocyte patterning and myelination. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5886093/