Alpers-Huttenlocher Syndrome (AHS) is a severe neurological disorder that also affects other parts of the body. AHS is known for having seizures that can often lead from one seizure to another. The disease affects 1 in 100,000 to 250,000 and 80% of individuals present symptoms within the first two years of their lives. The disease progressively destroys the individuals cognitive function, often debilitating an individual's motor skills. However the usual cause of death is often liver failure. The gene POLG (aka POLG1) is a major contributor to a multitude of genetic diseases and the most severe is Alpers-Huttenlocher Syndrome. The POLG gene encodes the α (alpha) subunit of the protein polymerase γ (gamma). This protein is necessary for mitochondria to replicate mitochondrial DNA (mtDNA), which is used to encode genes used in the mitochondria for energy production. POLG mutations can cause non functionality in the α subunit which leads to problems with mtDNA replication. Often mtDNA replication results in a decreased amount of functionable mtDNA which leads to a drop in the amount of energy the cell receives due to the mitochondria not receiving the correct proteins it needs for energy production that results from this. Although we know the phenotype caused by the POLG mutations, we do not know the physical affects the mutations have on the alpha subunit protein that causes disorder in normal function. Insight into this could lead to treatments of this disease as well as many other diseases associated with this gene.

My goal is to determine the cause of the variation phenotypic severity of POLG mutations**.** We will test our hypothesis that mutations found on the POLAc domain contribute to a more severe phenotype found in many disease that are related to the POLG gene.

1. Determine mtRNA levels via RNA-Sequencing in different POLAc mutants. We will use PCR to truncate different levels of our wild type protein. With one mutant having a partially truncated POLAc region, one with a complete knockout of that region and then another with the POLAc region intact but part of the middle linker region truncated. We will use this along with our RNA-sequencing techniques to measure our mtDNA level and use zebrafish models to see if we observe any seizures, using an EEG monitor.

2. Determine if proteins involved in mtDNA replication machinery are misregulated in POLAc mutants. Using the same mutants from the previous aim, we will look at the effects on other known proteins involved in the mtDNA replication process, specifically POLG2, C10ORF2 and SLC25A4. Again we will use RNA-sequencing to look at the relative levels of these interacting proteins. It's important to see how the mutation in our domain of interest affect these important interactors of the mtDNA replication.

3. Identification of key phosphorylation sites along the POLAc region using Net Phos. and then mutating those region of conserved phosphorylation across species. Then we can determine if there is a critical region along the POLAc region that causes our severe phenotypes and we will measure this by using zebrafish as our model and monitoring the motor function of the fish.

Determine This project will help us better understand Alpers-Huttenlocher syndrome and possibly lead us to more effective treatments and a better understand of the mitochondria as a whole. Along with this disease we will also be able to better understand other disorders associated with the POLG1 gene.