Tyrosinemia Type 1 is a rare autosomal recessive disease that directly impacts liver and kidney health, often leading to liver cirrhosis and hepatocellular carcinoma, while also displaying a vast number of other symptoms. [1] The three types of tyrosinemia are each classified by a different mutated gene. Type 1 is attributed to a mutation within the FAH gene that encodes for the enzyme fumarylacetoacetate hydrolase (FAA), which plays a large role in the catabolic tyrosine pathway and breakdown of tyrosine byproduct fumarylacetoacetate throughout the body, but specifically in the liver [2]. With this enzyme absent, patients have a buildup of this protein and its breakdown product succinyl acetoacetate (SUAC). This causes many complications, and while the effects on the kidney and liver have been well-studied and understood, *it is unknown exactly how the absence of a functioning fumarylacetoacetate hydrolase enzyme leads to an increase of hepatocyte apoptosis.*

My **primary goal** is to determine how *the FAA enzyme* facilitates the *cell cycle arrest and increased apoptosis* found in patients exhibiting liver cirrhosis. *Danio rerio* will be used as a model organism in this experiment due to its prominent similarities to the human liver in terms of function and structure as well as the added benefit of transparency and rapid development. [3] With this study, I **hypothesize** that the FAH gene regulates a key process involved in healthy cell progression and without this functioning gene, hepatocyte cells are stalled in the cell cycle, leading to increased apoptosis and in turn, cirrhosis of the liver. The **long-term goal** of this research is to further understand the mechanisms underlying this disorder to be able to effectively target symptoms with treatment drugs.

Aim 1 : Identify conserved amino acids necessary for healthy cell progression.
Hypothesis : Organisms with a mutated amino acid in the FAA domain will not progress as a healthy cell and will instead go through apoptosis at a checkpoint in the cell cycle. **Rationale :** Identifying conserved amino acids within the FAA domain necessary for healthy cell progression will allow for insight into certain mutations that cause this disorder, which can then be studied further to look at pathways and mechanisms that are involved with this disorder. Studying these mutated amino acids will allow for better drug treatment options that target these mutations found to cause the mutant phenotype. **Approach :** NCBI Blast will be used to identify homologs within humans and danio rerio. Using Pfam and Clustal Omega, an amino acid sequence alignment will be created and show many conserved amino acids. Selecting multiple conserved amino acids at random, these sites will be mutated in danio rerio using CRISPR. Once these mutations are induced, livers of the mutant zebrafish will be compared to the WT control group to evaluate phenotypic differences of the liver and verification by TUNEL assay will show apoptosis rates in each cell.

Aim 2 : Identify differentially expressed genes in WT and mutant FAH hepatocyte cells in zebrafish.
Hypothesis : FAH mutant hepatocyte cells will have different gene expressions than WT hepatocyte cells, specifically different regulation of genes involved in tyrosine metabolism. **Rationale :** By determining genes that are expressed differently in mutant zebrafish than in the WT, it will highlight different molecular mechanisms and pathways that are dysregulated in the disordered state. It also allows for identification of genes that can be used for therapeutic intervention. **Approach :** Using the mutant zebrafish from the previous aim, liver tissue cells are collected and ran through an RNA-sequencing machine to create a heatmap. This heatmap will then be sorted by GO for genes involved in the tyrosine metabolism pathway. Genes that are found to be upregulated in the WT and downregulated in the mutant zebrafish will be selected and knocked out using CRISPR. Phenotypic differences in the mutant fish liver will be compared to the WT control group. A TUNEL assay will further verify the levels of apoptosis in each mutant.

Aim 3 : Quantify differentially expressed proteins in WT and mutant FAH hepatocyte cells in zebrafish.
Hypothesis : Mutant FAH hepatocyte cells will have different protein expressions than WT cells, specifically in proteins that are involved in apoptosis of cells. **Rationale :** By quantifying proteins that have different expressions in mutant zebrafish than WT, insight is able to be gained about what occurs at the proteomic level. Evaluation into proteins that are important for cellular functions such as healthy cell progression and apoptosis will allow for treatment options that target these protein alterations for restoration back to normal function. **Approach :** Mutant fish from the previous aim that were found to have mutated phenotypes will be used in this experiment. The mutant cells will be grown in heavy media and WT cells in light media. Cells are harvested from each and lysed to extract proteins. Trypsin is then used to digest the proteins into polypeptides and both mutant and WT peptides will be mixed together. These will then be placed in a LC-MS/MS machine, which will yield protein expressions for each fish separated by light and heavy isotopes. Using CRISPR to knockout proteins with different expressions between the mutant and WT fish, phenotypic differences will be observed between the knockouts and WT control group. A TUNEL assay will further verify the levels of apoptosis in each knockout.

Through these aims, it will be better understood how the lack of a functioning FAA enzyme leads to increased apoptosis in hepatocyte cells. This will overall lead to deeper understanding of different mechanisms and pathways involved in this process and allow for future studies to target these pathways as treatment options for patients with Tyrosinemia Type 1, specifically those exhibiting liver cirrhosis.

References

1. Van Ginkel, W. G., Rodenburg, I. L., Harding, C. O., Hollak, C. E. M., Heiner-Fokkema, M. R., & van Spronsen, F. J. (2019). Long-Term Outcomes and Practical Considerations in the Pharmacological Management of Tyrosinemia Type 1. *Pediatric drugs*, *21*(6), 413–426. https://doi.org/10.1007/s40272-019-00364-4
2. (2024) *Tyrosinemia Type I*. Myriad Genetics. https://myriad.com/womens-health/diseases/tyrosinemia-type-i/
3. Shimizu, N., Shiraishi, H., & Hanada, T. (2023). Zebrafish as a Useful Model System for Human Liver Disease. *Cells*, *12*(18), 2246. https://doi.org/10.3390/cells12182246