**Specific Aims**

Stargardt disease leads to symptoms of colorblindness and gradual vision impairment leading to total vison loss, and effects an estimated one million people. Mutations to the ABCA4 gene contribute to a vast majority of cases of Stargardt disease, and about 5% of all retinopathies.1 The ABCA4 gene codes for a membrane transport protein that operates in rod and cone photoreceptor cells in the eye, transporting chemicals from the lumen to the outer disc membranes.2 This prevents the accumulation of toxic retinoid compounds that are likely the cause for the retinal degeneration seen in Stargardt disease. *There remains many factors of ABCA4 that are unclear, especially its role in overall retinol metabolism. Some studies explored some interactions involved in the process, but many regulatory interactions have not been investigated.*4,7

The **objective** of this proposal is to determine the role of ABCA4 in overall retinol metabolism. The results from this study could then be used to further understand the mechanisms that lead to chemical buildup in Stargardt disease. This will be performed through tests of the **hypothesis** ABCA4 affects overall retinol regulation and metabolism. The hypothesis was determined through the protein interactions involved with ABCA4, with many interactions and pathways that have been observed as coexpressing in other species.4,5 This is working towards the **long-term goal** of understanding retinol metabolism and its pathways. *Danio rerio* is the intended model species for this proposal, due to its fast retinal development time, similarity to human visual systems, highly conserved regions of ABCA4 to humans, and that it has been established as a quality species for studies into retinopathies.6

**Aim 1: Determine domains important for retinol metabolism between human and *Danio rerio* ABCA4 genes.**

**Hypothesis:** *Danio rerio* and human mutations in ABCA4 conserved regions will confer similar retinol metabolism phenotypes.

**Approach:** NCBI BLAST can be used to determine homologs between human ABCA4 and *Danio rerio* equivalents. This can be followed with Clustal Omega to identify conserved regions. The regions can then be disrupted with CRISPR-Cas9 and phenotypes can be analyzed and compared.

**Rationale:** Through analysis of phenotypes in *Danio rerio* ABCA4 conserved region mutations it can be determined how similar the mutant phenotypes are between human and *Danio rerio*, and which domains are critical for retinol metabolism. It can also orient us to differences we may have to account for between *Danio rerio* and humans.

**Aim 2: Determine modulation of retinol metabolism.**

**Hypothesis:** Retinol metabolism chemical pathways are disrupted in mutant phenotypes.

**Approach:** Common mutant and wild type *Danio* rerio populations can be produced, mutations developed via CRISPR-Cas9, and put through a wide chemical screen with some of the chemicals chosen using the PubChem website. The following phenotypes can then be analyzed in the mutant and wild type *Danio rerio* subjects.

**Rationale:** Analysis of the resulting phenotypes, both wild type and mutant *Danio rerio*, can elucidate both chemicals that disrupt retinol metabolism pathways, as well as chemicals that are important for normal operation of retinol metabolism pathways.

**Aim 3: Determine the expression of retinol metabolism genes through time.**

**Hypothesis:** ABCA4 and other retinol metabolism genes change expression over the lifetime of species.

**Approach:** *Danio rerio* retinal cells can be collected, with single cell types separated by tagging unique cell membrane features with fluorescing antibodies and isolated using fluorescent activated cell sorting. Cell types and expression can then be collected using single-cell RNA sequencing before analyzing using dimensionality reduction techniques such as principle component analysis and t-SNE.

**Rationale:** Analysis of expression could allow for an understanding of cell regulatory networks and response at different points in the life cycle. It would also allow for further understanding of ABCA4 in its regulatory network.

References:

1. Allikmets, R. (1997). Mutation of the Stargardt Disease Gene (ABCR) in Age-Related Macular Degeneration. Science, 277(5333), 1805–1807. doi: 10.1126/science.277.5333.1805

2. Quazi, F., & Molday, R. S. (2013). Differential Phospholipid Substrates and Directional Transport by ATP-binding Cassette Proteins ABCA1, ABCA7, and ABCA4 and Disease-causing Mutants. Journal of Biological Chemistry, 288(48), 34414–34426. doi: 10.1074/jbc.m113.508812

3. Zernant, J., Xie, Y. (A., Ayuso, C., Riveiro-Alvarez, R., Lopez-Martinez, M.-A., Simonelli, F., … Allikmets, R. (2014). Analysis of the ABCA4 genomic locus in Stargardt disease. Human Molecular Genetics, 23(25), 6797–6806. doi: 10.1093/hmg/ddu396

4. Molday, R. S. (2015). Insights into the Molecular Properties of ABCA4 and Its Role in the Visual Cycle and Stargardt Disease. Progress in Molecular Biology and Translational Science Molecular Biology of Eye Disease, 415–431. doi: 10.1016/bs.pmbts.2015.06.008

5. Auer, T. O., Duroure, K., Cian, A. D., Concordet, J.-P., & Bene, F. D. (2013). Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Research, 24(1), 142–153. doi: 10.1101/gr.161638.113

6. Fadool, J. M., & Dowling, J. E. (n.d.). Zebrafish models of retinal development and disease. Retinal Development, 342–370. doi: 10.1017/cbo9780511541629.019

7. Bryant, L., Lozynska, O., Maguire, A., Aleman, T., & Bennett, J. (2017). Prescreening whole exome sequencing results from patients with retinal degeneration for variants in genes associated with retinal degeneration. *Clinical Ophthalmology,* *Volume 12*, 49-63. doi:10.2147/opth.s147684