**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 *N*-retinylidene-PE 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 are over 800 confirmed mutations in the ABCA4 gene that are associated with the disruption of this process, and of these the most frequent mutation only occurs in 10% of cases.1,3 *There remains many factors of ABCA4 that are unclear, especially related to how it can be treated. Gene therapy has been successful in other retinal degenerative diseases, using gene delivery to replace recessive mutant alleles.4 There also is the option of more specifically focused gene editing, like CRISPR-Cas9, that have not been previously tested.*

The **objective** of this proposal is to determine the ability of gene therapy to recover normal function of the ABCA4 gene. The results from this study could then be used as possible treatment options for the many variants of mutations leading to Stargardt disease. This will be performed through tests of the **hypothesis** that gene delivery and gene editing can return function to ABCA4 and recover eye function from degenerative damage. The hypothesis was determined through the ability of recessive mutations of retinopathies to be repaired by gene delivery, and the ability of CRISPR-Cas9 to recover normal gene sequences.4,5 This is working towards the **long-term goal** of providing treatment for the varied forms of ABCA4 responsible Stargardt disease. *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 conserved amino acid sequences between human and *Danio rerio* ABCA4 genes.**

**Hypothesis:** *Danio rerio* and human mutations in ABCA4 conserved regions will confer similar 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 disease phenotypes are to human disease phenotypes. This can infer how similar recovery might function between our model species and humans.

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