**The Gavin R. Stevens Foundation and**

**The Foundation Fighting Blindness**

**Research Summary**

**Project Title:** Gene Therapy for *NMNAT1* LCA

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Ocular Genomics Institute, Mass Eye and Ear and Harvard Medical School

**Period of Support:** 2013 to 2016

**1. Background**

In 2011 several research groups reported that mutations in the *NMNAT1* gene cause Leber congenital amaurosis (LCA), accounting for about 5% of cases ([1-5](#_ENREF_1)). LCA is an early onset form of inherited retinal degeneration (IRD). Affected children have reduced vision and nystagmus (involuntary eye movements).

The finding that mutations in *NMNAT1* cause LCA is of interest for several reasons. First, it provides a target for efforts to develop treatments for IRDs, such as gene therapies. Second, it raises some interesting questions about retinal biology, the answers to which we hope will inform our efforts to develop treatment(s) for this form of retinal degeneration. For example, the *NMNAT1* gene encodes an enzyme that is needed for synthesis of nicotinamide adenine dinucleotide (NAD) inside cells. NAD has multiple important roles in cell function, including helping capture energy from nutrients, and regulation of the function of several types of proteins. As a reflection of the importance of NAD in cell function, there are three versions NMNAT in cells; NMNAT1 is located specifically in the nuclei of cells ([6](#_ENREF_6)). Since NAD is needed in the nuclei of all cells, it is not clear why mutations in *NMNAT1* affect the retina but not other parts of the body.

Data from studies performed to date suggest that the retinal disease which results from mutations in the *NMNAT1* gene is caused by reduction NAD production by NMNAT1 in the nuclei of retinal cells. Unfortunately, the other versions of NMNAT, called NMNAT2 and 3, cannot compensate for the loss of NMNAT1 function ([7](#_ENREF_7)). The goal of our research is to test the idea that restoration of NMNAT1 function to normal levels via gene augmentation therapy can be beneficial for patients affected by *NMNAT1*-associated retinal degeneration.

**2. Research Program**

A. *Nmnat1* Mutant Mice

In order to test the use of gene therapy for treating NMNAT1-associated retinal degeneration, we first had to identify a model system to use for our research studies. Through collaborations with Dr. Mike Bowl from the Medical Research Council (MRC) center for mouse genetics in Harwell, England and Patsy Nishina at the Jackson Labs in Maine, we have identified and characterized two different mouse models of NMNAT1-associated disease.

First, with Mike Bowl’s help, we identified mice with a mutation in NMNAT1 that changes the 9th amino acid in the protein from Valine to Methionine. This mutation is abbreviated as Val9Met, and has been found in several patients affected by *NMNAT1*-associated LCA. Like the patients, the *Nmnat1*-Val9Met mice experience rapid retinal degeneration, with reduction of retinal function and loss of retinal cells detected within 4 months. These data suggest that the light sensitive or photoreceptor cells of the retina are the first cells to be affected to reduced NMNAT1 function ([8](#_ENREF_8)).

Second, investigators in Patsy Nishina’a lab found mice with an Asp243Gly mutation in NMNAT1. The Asp243Gly mutation has not yet been identified in patients with retinal degeneration. These mice also have retinal disease, with a slower rate of degeneration than the Val9Met mice ([8](#_ENREF_8)).

B. NMNAT1 Gene Therapy

With good models of *NMNAT1*-associated disease in hand, we are now working on developing gene augmentation therapy for *NMNAT1*. For these studies, we are using delivery vectors derived from Adeno-Associated Virus (AAV). AAV is the gene delivery system that is being used in multiple other gene therapy studies, including the clinical trials of gene therapy for *RPE65*-associated retinal degeneration and CHM-associated retinal degeneration (choroideremia) ([9-15](#_ENREF_9)). .

Unlike the *RPE65* and *CHM* studies in which the therapeutic gene needs to be delivered to the retinal pigment epithelial (RPE) cells of the retina, we need to use AAV to deliver NMNAT1 to the light sensitive or photoreceptor cells of the retina. We are fortunate to be doing these studies in collaboration with Dr. Luk Vandenberghe, an expert in the use of AAV for gene therapy.

For these studies, we are making and testing several AAV vector systems for *NMNAT1*. The elements we are evaluating include different promoters, which drive the expression of the therapeutic *NMNAT1* gene in retinal cells and different vector serotypes, which modulate the types of cells infected by the viruses. All the AAV vectors we are testing have been made with a synthetic human *NMNAT1* gene that can be used in future human studies. Vector preparations are generated and purified in the Ocular Gene Transfer Core Facility of the OGI using established techniques ([16](#_ENREF_16),[17](#_ENREF_17)).

**3. Summary**

We are hopeful that the ongoing studies will provide evidence that gene augmentation therapy for *NMNAT1*-associated retinal degeneration can be beneficial. Our goal is to identify the best gene therapy approach to bring forward towards human clinical trials. If our initial attempts at gene therapy in the *Nmnat1* mutant mice are not successful, we are hopeful that improved understanding of the mechanism by which mutations in *NMNAT1* lead to retinal degeneration will help us identify additional approaches which can ultimately be used for treating patients with this form of LCA.

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