**PhD studentship: Development of CRISPR gene therapy for Stargardt disease**

**Start date and grant duration: Oct 2020, 36 months**

**Principal Investigator: Prof Robert MacLaren, Nuffield Department of Clinical Neurosciences, University of Oxford**
**PhD student: Elena Piotter.**

Elena completed her undergraduate degrees in International Relations (BA) and Global Disease Biology (BSc) at the University of California- Davis. She moved to the Netherlands where she completed a post-graduate course in molecular biology (MSc). As part of this degree, she undertook a project in Professor MacLaren's lab over 10 months, resulting in the submission of a conference abstract to the American Society of Gene and Cell Therapy. She was subsequently awarded her current DPhil position within the MacLaren research group.

During the first year of her studentship, Elena has presented to the Retina UK community at a local peer support group and as part of the charity's webinar series. Elena also undertook the Peak District Challenge and raised a significant amount of sponsorship for Retina UK.

Retina UK is co-funding this project with the Macular Society.

**Date of report: November 2021 (12 month report)**

**Project background**

This project aims to look into a potential new method for treating Stargardt disease and other conditions where existing gene therapy approaches may not be possible.

Stargardt disease is caused by mutations in the ABCA4 gene, a very large gene that does not fit inside the viral delivery system typically used to send healthy copies of genes into cells during gene replacement therapy. Because of this issue, there are currently no viable gene therapy strategies for Stargardt disease.

In recent years, new molecular tools for gene editing have been created; these are known as CRISPR-Cas systems. They can be used to correct mutations either by direct editing of DNA, which introduces permanent changes to the gene, or by editing RNA, in which the changes exist only transiently for the life of the RNA molecule. (Cells use RNA to make copies of the DNA blueprint and then carry the genetic instructions contained within it from the centre of the cell, out to the cell's protein building machinery. Once the protein has been constructed, the RNA is broken down.)

The focus of this project is to compare the efficiency and safety of DNA and RNA editing systems for targeting mutations in the ABCA4 gene. Recent advances in CRISPR technology have allowed for correction of a particular single letter spelling mistake that occurs relatively frequently in disease-causing ABCA4 mutations and can be corrected in either DNA or RNA. The project will therefore compare rates of correction of this mistake (efficacy) and occurrences of unwanted edits (safety) between DNA- and RNA-editing systems.

The project will use a step-by-step approach, initially testing and adjusting the CRISPR systems in cultured cells, before moving on to test the optimised systems in a specially generated mouse model. The ultimate goal is to develop an efficient and safe editing system for the treatment of Stargardt disease.

**Progress to date**

Analysis of pathogenic ABCA4 variants

To gain a better understanding of the clinical relevance of CRISPR-Cas editing for Stargardt disease, the researchers undertook a systematic review of all disease-causing ABCA4 mutations across three international patient databases. They found that an encouraging proportion of the mutations were near to special sequences of genetic code, called PAM-sites, which enable the molecular editing machinery to work properly to correct the mutation.

In addition, the team screened three published patient cohorts from Germany, Denmark and China for a population-relevant analysis. This also produced promising findings: between 44 and 76% of the patients had at least one mutation close to an “ideal” PAM-site, meaning that these patients would potentially experience clinical benefit from current CRISPR-Cas techniques.

Preparation for in vitro testing

In order to develop and assess CRISPR approaches, the team will need to undertake extensive testing and optimising in cell lines (*in vitro* testing). To carry out this work, the researchers must first select and create relevant mutations in ABCA4 for targeting, consider different CRISPR construct designs, and design and test guide sequences that will enable the CRISPR tools to target the mutations.

During the first six months of the project, the researchers selected appropriate mutations to focus on (see six month report). They have since finished creating plasmids (short strands of DNA) containing these mutations, and have then inserted the plasmids into cultured human cells (Human Embryonic Kidney 293T cells), which use the newly introduced genetic code to produce ABCA4 protein. The amount of protein and the effects of the mutations on the protein molecules can then be examined using a laboratory technique called western blotting; this has shown that the cells are generating ABCA4 protein but that the molecules are shortened or altered in line with the expected effects of the mutations.

The team will now move on to test and optimise their CRISPR-Cas constructs, assessing their ability to edit the mutant plasmids in the HEK cells, before eventually progressing to *in vivo* work in the specially developed mouse model designed at the beginning of the project (see six month report).

**Thank you to everyone who supports our work and makes projects like this possible.**

For further information about this and all the projects Retina UK is funding, please contact Kate Arkell, Research Development Manager, on kate.arkell@RetinaUK.org.uk. For information about supporting this or other projects, please contact Alice Capper in the Fundraising team on alice.capper@RetinaUK.org.uk, or call 07841 481423.