Development of CRISPR gene therapy for Stargardt disease.
Six month progress report.

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Retina UK is co-funding this PhD studentship project with the Macular Society. A profile of the student employed on the grant, Elena Piotter, can be found at: www.RetinaUK.org.uk/research/researcher-profiles/researcher-profile-elena-piotter/ . Elena is keen to engage with the inherited sight loss community and has already presented at a Retina UK online support meeting. She will also be undertaking a fundraising challenge event for Retina UK this year.

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:

Design of the mouse model

An appropriate mouse model with a disease-causing ABCA4 mutation will be required to assess the safety of the CRISPR systems as well as their ability to impact pre-clinical endpoints such as restoration of vision. Given the relatively long timeframe required to generate a new transgenic mouse line, the design of the model has been an early priority for the project.

To find an optimal target mutation, the researchers looked through all known disease-causing ABCA4 mutations and selected an ideal candidate that could be corrected in either DNA or RNA with the proposed CRISPR tools. This mutation and its surrounding sequence from the human genetic code will be introduced into the mouse model so that the mice can be used to test the efficiency and safety of human-relevant CRISPR systems.

It is anticipated that the mouse model will be generated within 1 to 2 years, at which point the researchers will characterise the effects the ABCA4 mutation on the animals' eyes and begin testing the optimised editing systems.

Preparation for cell-based work

Before assessing their CRISPR systems in the mouse, 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.

The team has decided on four ABCA4 mutations to study. One of these is the mutation selected for the mouse model, which will allow appropriate preparation for the later work in the mouse, with the other three being selected on the basis of their prevalence in human disease and the ease with which editing outcomes can be assessed. Combined, these four mutations will allow comprehensive optimisation of the CRISPR systems *in vitro*. The researchers have created the mutant ABCA4 by inducing specific changes at the relevant point in the genetic code, and they are going on to produce multiple copies of the mutated gene for insertion into cultured cells.

The researchers are also generating the CRISPR tools they will need, which will be carried into the cells within an empty virus. They are using currently available CRISPR options for the time being, but are likely to expand the range of CRISPR systems they test over the course of the project as new options emerge.

The researchers have already designed a number of guide sequences for each mutation to enable the CRISPR systems to target the right section of genetic code for editing. The efficiency of these will be compared to a non-targeting control.

Upcoming experiments in the cell line will be critical in identifying the best guide and CRISPR system designs with which to move forward.

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 Research Development Manager Kate Arkell, on kate.arkell@RetinaUK.org.uk. For information about making a donation towards this or other projects, please contact Alice Capper on alice.capper@RetinaUK.org.uk, or call 07841 481423.