An investigation into non-viral gene therapy using S/MAR vectors for Usher syndrome.

Final project summary – prepared February 2022.

Usher syndrome is a genetic condition which causes hearing impairment and progressive sight loss, and is often diagnosed in childhood. It accounts for 50% of inherited deaf-blindness worldwide, with an estimated 400,000 people living with the condition.

Faults in the *USH2A* gene are the most prevalent cause of Usher syndrome, and a gene therapy approach that supplies healthy copies of *USH2A* is a potential route to treatment. However, for most gene therapies, the healthy gene is packaged into a virus for delivery into the diseased cells; this is not possible for *USH2A* because it is a very large gene and will not fit into a virus.

Principal investigator: Professor Mariya Moosajee
Research institution: UCL Institute of Ophthalmology and The Francis Crick Institute
Project dates: March 2018 – December 2021.

Project aims

Professor Moosajee's project was focused on developing a non-viral gene delivery system (known as a vector) with sections of human DNA called scaffold/matrix attachment regions (S/MAR), which can accommodate *USH2A* and enable its delivery into cells. Apart from being able to package very large genes, S/MAR vectors also have a number of other benefits, including a reduced risk of immune response and unwanted mutations. Successful completion of this project could therefore lead to safe and effective therapies for other retinal conditions such as Stargardt disease, which also involves a large gene, and many other genetic eye conditions regardless of gene size.

Developing the vector

At the beginning of the project, Professor Moosajee's team had developed several prototypes of the S/MAR vector for *USH2A*. This proved challenging due to the length of the gene, but the team eventually achieved this by breaking the gene into fragments and slotting them into the package one by one like a jigsaw. However, on proof-reading the entire genetic sequence for the packaged *USH2A*, the researchers found one single letter spelling mistake. They have now rectified this and have been able to confirm that they have the correct *USH2A* sequence successfully inserted into the S/MAR vector. They have also included a variety of special signals (known as promoters) that can encourage the gene to be switched on, either in most cells or specifically in retinal cells. The vector itself is a key output from this project, and is now available for future studies and testing in model systems.

Developing models for testing

Over the course of the project, the team made good progress with testing their approach in cell and animal model systems.

They took skin biopsies from two Usher syndrome patients with different *USH2A* mutations and used the skin cells, as well as human embryonic kidney (HEK) cells, to test the efficacy and optimal dosages of their various S/MAR *USH2A* prototype designs. The team was able to

detect usherin, the USH2A protein, in both cell types, suggesting that the vector was doing its job of delivering functional genetic code into the cells.

Professor Moosajee's team converted some of the Usher patients' skin cells into stem cells and then differentiated them into retinal cells to create a simple "retina in a dish", sometimes called a retinal organoid, or eye cup. These disease models were carefully characterised so that the team could fully understand the differences between the USH2A and healthy retinal organoids. In particular, the researchers noted that special extensions of the retinal cells, known as cilia, were much shorter in the USH2A eye cups compared to healthy controls.

Going forward, cilia rescue could be a good functional marker of response to treatment in these models. However, on this occasion, the team found that the gentle electrical pulses that they were using to help the vector enter the cells were in fact causing damage and negatively affecting cilia formation. Prof Moosajee will continue investigating and optimising vector delivery methods, including the use of lipid nanoparticles, to avoid this issue in future.

Professor Moosajee has used gene editing to introduce mutations into the *USH2A* gene of zebrafish, which consequently lacks the usherin protein. During the course of this project, her team has undertaken (and published) a detailed investigation of the unique *USH2A*-related characteristics of this model during its early development, so that they can use appropriate measures of any improvement when the new therapy is used in the fish.

The researchers have optimised a method to inject zebrafish embryos with the new S/MAR USH2A vector at the earliest stages of development, and both healthy and ush2a mutant fish have been treated at various doses. The vector appears to be leading to successful use of the USH2A genetic code for at least 12 months as the fish mature, as demonstrated by the presence of a fluorescent protein indicator and USH2A mRNA in the fish retinas.

Summary

This has been an exciting and successful project overall with several important outputs, including fully characterised fish and cell-based models of USH2A disease, as well as the new vector itself. The project has shown proof-of-principle that the vector can restore USH2A expression in these models and is a potential mutation-independent gene therapy approach for USH2A and other large disease-causing genes. Prof Moosajee will continue to progress her work with the vector, including investigating better ways of delivering it to retinal cells.

We are so grateful to everyone who has generously supported this important investigation. Your support is helping us draw closer to a world where children and adults affected by Usher syndrome can access treatments.

If you would like further information about our medical research projects or supporting our work through donations, please contact: Deborah Laing, Head of Fundraising, deborah.laing@RetinaUK.org.uk, 07841 004564.