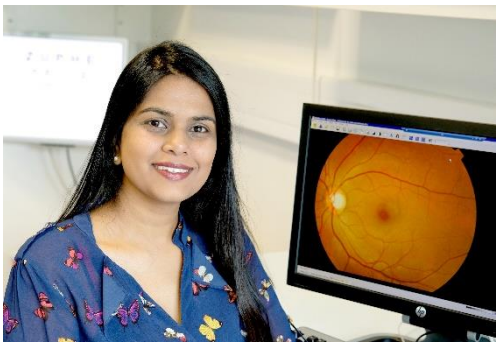
An investigation into non-viral gene therapy using S/MAR vectors for Usher syndrome.

Summary of second year report (published March 2020).

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: Dr Mariya Moosajee
Research institution: The Francis Crick Institute
Project dates: March 2018 – March 2021

Project aims

Dr Moosajee is developing a non-viral gene delivery system (known as a vector), using pieces of human DNA called scaffold/matrix attachment regions (S/MAR), to encase *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 introducing cancer-causing 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 possibly other genetic conditions which do not involve the eye.

Developing the vector

At the beginning of the project, Dr 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. 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.

Developing models for testing

Meanwhile, the team has made progress with testing their approach in cell and animal model systems. They have taken skin biopsies from two Usher syndrome patients with

USH2A mutations and used the skin cells to test their various S/MAR *USH2A* prototype designs. One way to do this is to use antibodies that bind to the *USH2A* protein produced by the newly introduced gene. Unfortunately, the antibodies the researchers tried initially didn't work in the skin cells. Dr Moosajee has therefore been in contact with other international teams working on Usher syndrome, who have shared their antibodies with her; these are now being tested.

Meanwhile, there is other evidence that the gene therapy is working in the skin cells; the S/MAR vectors include a fluorescent green "tag" as a surrogate marker of USH2A protein production, and green fluorescence can be seen in the skin cells after vector delivery.

The other scientists have also suggested that the antibody approach might work much better in photoreceptors than in skin cells. Dr Moosajee's team has already turned some of the Usher patients' skin cells into stem cells and started the process of differentiating them into retinal cells; this has been successful so far. Once these cells have developed sufficiently into a basic "retina in a dish"; the researchers will study their characteristics before introducing the S/MAR vector to them and measuring any effects.

Dr Moosajee will be using zebrafish with *USH2A* mutations to test the S/MAR gene therapy approach in an animal system. 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. In the meantime, healthy fish have been injected with the S/MAR vector, which appears to be working in their retinas.

Summary

The researchers have successfully produced a S/MAR *USH2A* vector and are overcoming any issues that have arisen during testing. They will spend the final year of the project establishing the efficacy of the vector in the human cell and fish models.

Successful completion of this project will allow Dr Moosajee and her colleagues to take the new vector forward for further testing in the hope that it can eventually progress to a clinical trial. This would be for Usher syndrome in the first instance, but there is potential to provide therapeutic options for many patients who are not candidates for conventional viral gene therapy.

Thank you so much to everyone who is generously supporting this important investigation.

If you have any questions, please contact: Kate Arkell, Research Development Manager, kate.arkell@RetinaUK.org.uk.