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

First year report (published March 2019)

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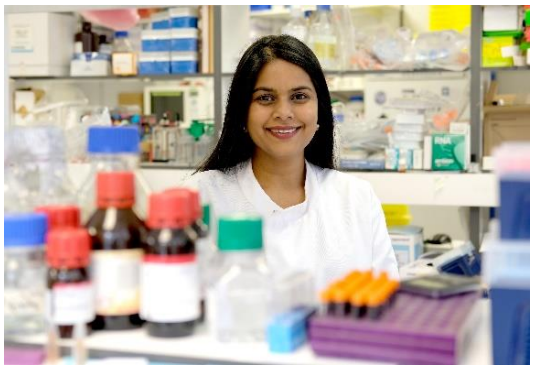
Start date and duration: 22nd March 2018, three years



Dr Moosajee in the lab

Usher syndrome is the most common cause of deaf-blindness worldwide, with faults in the *USH2A* gene being the most prevalent cause. 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 simply will not fit into a virus.

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.

Developing the vector

During the first year of the project, Dr Moosajee's team developed several prototypes of the S/MAR vector for *USH2A*. This proved challenging due to the length of the gene – almost 16,000 letters of the genetic code need to be packaged into the delivery system. This has not been done before and required several strategies. The team eventually achieved this by breaking the gene into fragments and slotting them into the package one by one. This methodology has proved successful overall. However, on proof-reading the entire genetic sequence for the packaged *USH2A*, the researchers found one single letter spelling mistake, which they are now working to correct before they can go on to look at the impact of the gene therapy on diseased cells.

Developing models for testing

Meanwhile, the team is making preparations for testing their approach in cell and animal model systems. They have taken skin biopsies from two Usher syndrome patients with *USH2A* mutations and painstakingly turned some of the skin cells into stem cells. These can eventually be further converted into retinal cells, which will contain the disease-causing mutation and can be used to assess the effects of healthy *USH2A* delivered by S/MAR vectors. For now, the researchers have used the skin cells to work out which of their S/MAR prototypes is the most efficient delivery system.

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 a detailed investigation of the unique *USH2A*-related characteristics of this model during its early development, so that they can work out the best ways of measuring any improvement in structure and function of the retina and ear when the new therapy is used. They have also optimised their technique for injection of the vector into the fish.

Summary

The project is proceeding in line with budget and is largely on target against predicted milestones. The researchers have a clear plan for correcting the small spelling mistake in the S/MAR packaged *USH2A* and are putting this into action now. They will then move on to investigate the effects of using S/MAR to deliver healthy *USH2A* into the stem cell model and the zebrafish.

The successful generation of a safe and effective gene delivery system that can hold large genes such as *USH2A* will be a significant advance in the field of gene-replacement therapy and provide therapeutic options for many patients who are not candidates for conventional viral gene therapy.

A huge thank you to the individuals, businesses, trusts and charitable foundations who have supported this important investigation so far.

Your support is having a huge impact on our research team's understanding of this life-changing condition.

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