UCL

Non-viral Gene Therapy for Usher Syndrome

A Report for CUREUsher
October 2023

Novel, pioneering therapy

We are deeply grateful for CUREUsher's support of Professor Mariya Moosajee's research into novel treatments for Usher syndrome.

The following is an update from Dr Moosajee on the work of her and her team.

Understanding USH2A

Usher syndrome is an inherited genetic disease that affects hearing, vision, and often balance. Though scientists have identified nine genes that cause Usher syndrome, USH2A is the gene most commonly responsible.

USH2A is a very large gene, with over 790,000 letters of the DNA code, written using a 4-letter alphabet. We all inherit two copies of each gene. One from our father, and the other from our mother. If the DNA has a spelling mistake (also known as a mutation) in the code written with a 4-letter alphabet, the gene may lead to the development of a condition or disease. Therefore, if we inherit two USH2A copies with spelling mistakes, we are likely to develop Usher syndrome. So far, more than 1,500 different spelling mistakes in the USH2A gene have been described.

Gene augmention therapy

Gene augmentation therapy is a treatment strategy that aims to bring a new healthy copy of the gene to the cells that need it. This strategy would work independently from the spelling mistakes so that patients with any genetic change or mutation in USH2A could benefit. Gene therapy usually uses viruses as vehicles to deliver the gene to the targeted cells. However, the USH2A gene is much too large for the viruses used in this process.

In this study, we designed a circular human DNA containing a healthy USH2A gene copy and delivered it into USH2A patient skin cells and an USH2A zebrafish model. We demonstrated that this circular DNA was being read by the cell and producing usherin, the important protein for the light-sensitive cells of the retina (photoreceptors).

This is the first proof-of-concept reported of an all-in-one USH2A gene therapy vector. The next step is now to test these circular DNA vehicles in more complex animal models and with solutions to allow its optimum delivery into photoreceptors.

Work in progress

We are now planning to test this gene delivery system on models closer to humans, and are focused on four objectives.

1. We have taken a skin biopsy from a patient with Usher syndrome, and have converted the skin cells into stem cells. The team is now growing the patient's own retinas in a dish using stem cell technology, and has created a video of this process.
2. Once we have grown the retinas in a dish, we will study these closely and then plan to apply the non-viral gene therapy to see if it helps the retina recover (see Figure 1 for characterisation of WT retinal organoids which shows expression of USH2A, whirlin, and rhodopsin. This needs to be completed for the disease group).
3. In order for the non-viral gene therapy to enter into human retina, it needs to be packaged in a solution, called nanoparticles, that allows it to penetrate into cells. We have been working with several collaborators from around the world to test various nanoparticles to assess safety and effectivity. Preliminary experiments have shown that some nanoparticles work better than others, so the team are taking those with the best chance of success and are validating them further in patient cells (see Figure 2).
4. I have formed a collaboration with Dr Yannis Paulus and Dr Dongshan Yang from the University of Michigan. The US group have generated a rabbit model of Usher syndrome with an USH2A genetic change. Once we have optimised the nanoparticle delivery system in objective 2, we will work with the US group to deliver the non-viral gene therapy to the rabbit retina, in the exact same way we would deliver this to patients. This work will provide safety data and information on whether there is successful gene replacement.

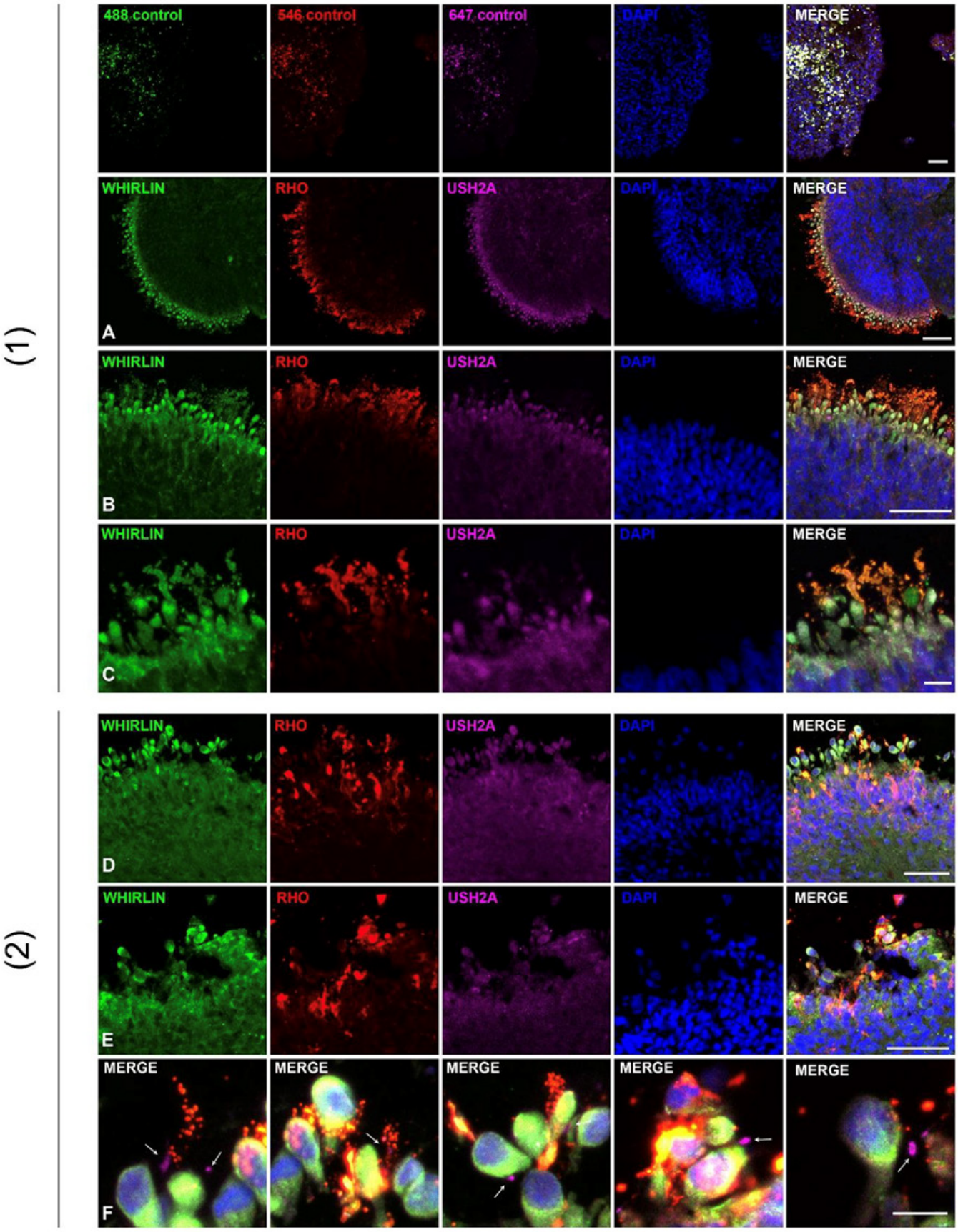

Figure 1. Forty-two-week-old wild-type retinal organoids immunostained to detect Usher proteins. Two 42-week retinal organoids with preserved lamination were antibody stained for usherin (magenta), whirlin (green) and rhodopsin (red). Arrows highlight spot-like usherin signal in the photoreceptor cells. Secondary antibody-only controls are shown in the top panel. Scale bars = 50 µm (A, B, D, E) and 10 µm (C, F).

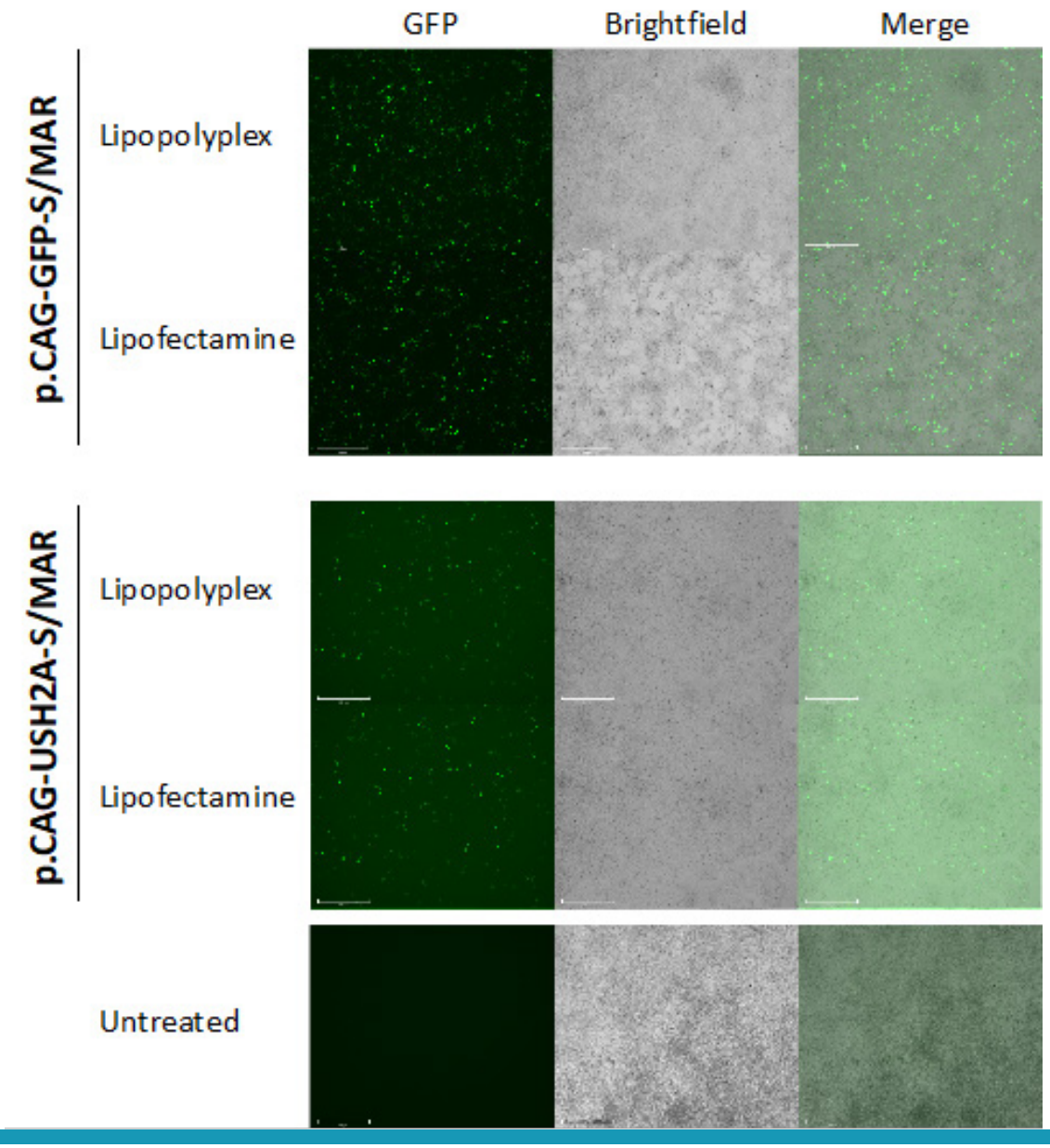

Figure 2. Transfection of non-viral S/MAR plasmids into human embryonic kidney 293 (HEK293) cells using receptor-targeted lipo-peptide nanocomplexes (lipopolyplex). HEK293 cells were seeded in a 24-well plate, 2x105 cells/well were transfected after 24 hours with 500 ng pDNA/well. Cells were imaged 24 hours later. Chemical transfection reagent Lipofectamine was used as a positive control (this is too toxic to use on patients but works well for cell culture). Green fluorescent protein is seen where the plasmid has successfully entered the cell and is producing protein. This shows that the lipopolyplex is working successfully. We are now testing this in human patient fibroblasts and will move to mouse models.

Above: Professor Mariya Moosajee

Advancing understanding by identifying biomarkers

The retinal degeneration associated with Usher syndrome is relatively slowly progressive. In a clinical trial setting patients would need to be followed up for at least 2 years to be confident of a change in disease progression from retinal imaging. In order to detect a biomarker that may give an indication that an orally delivered drug is working at an earlier stage, and also to provide more insights into the effect of Usher syndrome on the rest of the body, we have commenced a study to look into all the metabolites (small molecules like amino acids, lipids, peptides, carbohydrates, etc.) in the blood of Usher patients (those with USH2A) versus healthy individuals.

Studies like this have been undertaken successfully for a number of other retinal diseases, so we do expect to detect significant differences. We have recruited 10 patients so far and need blood samples for another 15 patients so that we can begin the process of whole metabolomic screening.

Plans for the next year

My team and I are hugely grateful for CUREUsher's continued support which is making all of this possible and is powering our vital research to find new treatments for Usher syndrome.

With adequate funding, our projects will continue to push forwards over the next year. Funding will support the work of Dr Maria Toms, who returns from maternity leave in January 2024.

The main goals for 2024 are to:

1. Optimise the lipopolyplex nanoparticles in patient fibroblasts and retinal organoids;
2. Assess safety/toxicity in wild-type mouse and rabbit models;
3. Commence non-viral gene therapy in Usher rabbit models;
4. Identify a systemic biomarker for Usher patients for use in clinical trials.

If further funding becomes available, we would like to begin to expand the disease models we are generating to include patients with other genetic forms of Usher syndrome so that our research can be more inclusive and applicable. There is much to learn about the disease mechanisms and potential shared disease pathways for the development of targeted therapies.

The work being done in the Moosajee Lab brings us ever closer to understanding Usher syndrome. Your partnership allows our team to continue developing much-needed non-viral therapy for patients with this rare but debilitating genetic disease.

We are deeply grateful for your continued dedication and commitment to Dr Moosajee's work aiming to change the lives of those with Usher syndrome. Your commitment brings us closer to a world with improved vision, hearing, and hope.

Thank you.

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