Hello. My name is Gwenaëlle Géléoc and this is "USH Talks." I am an assistant professor at Boston Children's Hospital and Harvard Medical School. And today, I'm going to report on work that we've done recently in the lab looking at gene therapy for Usher syndrome type 1C.

In the lab, we've been studying hearing and deafness, and we've been looking at the role of different genes that are important for the sensory cells and the neurons of the auditory organ and of the vestibular organ to function. In particular, I've been interested in Usher syndrome and more specifically recently, on Usher syndrome type 1C. And the mutation that is common in patients that are found in Louisiana of Acadian descent.

This mutation is called 216 G to A and a mouse model of this mutation was developed by Jennifer Lentz and Bronya Keats many years ago. They had characterized this model, and we furthered characterized it looking at hearing balance, histology, and physiology. We then developed gene therapy vectors specific for this mutation.

To evaluate the therapeutic benefits, we performed auditory and balance testing, performed cellular physiology assay looking at imaging of the sensory cells, and we also performed molecular work. So this gene is expressed--the Usher 1C gene is expressed in the auditory organ and the vestibular organ from the base to the apex of the cochlea, as well as here in the vestibular organ and in the saccule, utricle and the Ampulla. It's expressed in all types of sensory cells and here is shown a cross-section of the auditory organ, where we see that harmonin is expressed in outer hair cells and inner hair cells.

This is also seen in the scanning electro micrograph here, that also depicts the structure of the tip of the hair cells, which is called a stereocilia structure or hair bundle, which is essential for sensing the displacements of the liquid that is transmitted through sound waves to the auditory organ.

So the goal was to develop specific vectors for this gene. So we prepared gene sequences, which we packaged into a vector that was developed by Luk Vandenberghe, and transferred these vectors through the round window of the mouse. We have demonstrated that the vector we were using was excellent in targeting inner and outer cells, and this is seen here in green. This is a vector driving the green fluorescent protein, so we can see that the vector is excellent in driving expression of a gene to a specific target.

So we package the sequences in this vector. We injected the mice after birth and we let them recover. Six weeks later, we first observed the mice in an open field, which is an open chamber of 42 centimeters. Here in the upper panel, you can see mice that are freely moving in an open chamber.

On the right is a control mouse. On the left is a mutant mouse. And what you will notice is what the mutant mouse

is performing many circling motions and is moving quite frantically around the chamber, the control mouse is more calm and is exploring mostly the periphery of the chamber, and is not performing any of the circling motions.

Now, if we look at a mouse that's been treated after birth and six weeks after, and observed the mouse's movement along the chamber, we can see that their motion resembled a lot more the control mouse. We associate this recovery to a recovery of the vestibular function and the balance function.

We also performed auditory brain stem responses. This is done in anaesthetized mice, which are allowed to recover after the recording. We apply sounds from 10 decibels-- a whisper-- to 120 decibels-- a very loud sound. In control mice, we can see that we can record electrical responses at sounds as low as 20 decibels. The response increases in amplitude as you increase the sound level.

In mutant mice, despite increasing the exposure to up to 110 decibels, we do not see an electrical response, showing that the mutant mice are fully deaf. In the treated mice now, we can observe electrical responses as low as 30 db and clearly by 35 db here. And the response again increases in amplitude as we increase the sound exposure.

So we can see that we've successfully rescued hearing in those mice to a level that's very close to the control mouse. So this is unprecedented. Such a recovery has never been seen before, so we're really thrilled by this result.

We then performed cellular physiology. We dissected the cochlea, put it in our inner recording chamber under a microscope. And we can record from the sensory cells-- from each cell-- while displacing the sensory hair bundle because of this. And this basically mimics what would occur during the provocation of a sound wave.

When we apply a stimulus, we displace the bundle towards the stereocilia. We see an increase in the current that corresponds to the ions coming into the cells, which would eventually depolarize the cell. If we apply different levels of stimulus, we can see an increase in the current gradually with increasing stimuli.

When we look at the mutant mouse, we can see that the currents have been drastically reduced. However, after treatment, we can see that we've recovered the currents close to the control mouse. So this shows that we've rescued the sensory hair cells and this likely has led to the recovery of the auditory and vestibular function.

Furthermore, if we look at imaging and the structure of the stereociliary bundle, we can see in the control mice, we have normal structures. And stereociliary bundle that possesses three rows of stereocilia, I colored them here to show you the three different rows of stereocilia. In the mutant mice, the structure is quite disorganized. A lot of cells have died, and we've completely lost these nice structures that we've seen in the control mice.

What happened after treatment? We can see that we have recovered a lot of sensory cells, and the three rows of stereocilia are now present in the mice that have been treated after birth. So we've recovered some morphology of the stereociliary bundle.

Finally, we did the molecular work, and what we did here is look at expression of RNA, which is a genetic message that will lead to the expression of the protein. We can see that there is mostly aberrant RNA detected in the mutant mouse. After treatment, we can now detect the normal RNA encoding for the normal protein.

What about the protein itself? We used a technique called immunohistochemistry. And in green, we can see the antibody that labels specifically harmonin. In red, we have the actin just to show where the tissue is. You can see that expression of harmonin is normal in the control mice, it's absent in the mutant mice, but recovered after treatment.

So in summary, we have developed gene therapy vectors specific for the Usher 1C mutation-- the 216 G to A Usher 1C mutation. We have seen rescue of auditory and balance function. We observed rescue of mechano-sensation in the sensory hair cells. We've increased survival of sensory hair cells and observed recovery of hair bundle morphology. We also see normal RNA and protein in the treated mice.

So before I conclude and thank our sponsors and the people who've done this work, I just want to say how grateful I am for being given the opportunity to work on this project. And we are extremely encouraged by the result we've seen. At the same time, we want to be very cautious. It's going to take a lot more work to know whether any of the work that we've done is eventually going to be applicable for human treatment.

In particular, we want to know whether this work could be translated to larger animals before we can move onto humans. The work was sponsored by Boston Children's Hospital, the Kids Be Kids Foundation, the Foundation Fighting Blindness, as well as the Foundation Bertarelli. Many people have taken part in this work. In my lab, Bifeng Pan, a physiologist, Charlie Askew, Alice Gavin, Carl Nist-Lund, Selena Heman-Ackah, Yukako Asai and Jeffrey Holt, who is my colleague and collaborator-- as well as collaborators at LSU, Jennifer Lentz, Chicago Medical School, Michele Hastings and Francine Jodelka-- at the Mass Eye and Ear in Boston, Luk Vandenberghe who developed the vectors that we used for this study-- and HMS, our medical school in Boston, Artur Indzkykulian who performed the scanning electro-microscopy Thank you for your attention.