Usher Syndrome Coalition | USH1B Update, Shannon Boye, Ph.D.

Good morning. I'd like to thank the Usher Syndrome Coalition for inviting me to speak today. For those of you that don't know me, my name is Shannon Boye. I'm an associate professor of pediatrics and the associate chief of the Division of Cellular and Mollecular Therapy at the University of Florida. And I'm excited today to present to you an update on the research that we've been doing into USH1B.

And before I continue, I just want to give a short shout-out to Miss Lia who's photographed on the left. She is one of our most inspiring USH1B patients, and I really appreciate that her mom and dad allowed us to present her picture at the start of this slide show. So as everyone here knows, Usher syndrome is the most common condition affecting both vision and hearing. It's inherited in a recessive pattern and it affects between about 7 to 14 out of every 100,000 people.

People with Usher syndrome develop retinitis pigmentosa, bilateral deafness or hearing loss, and can sometimes have vestibular or balance problems as well. And now, out of all of the forms the Usher syndrome that one can have, Usher syndrome type 1B is one of the most common and most severe subtypes. It actually accounts for the majority of all USH1 cases and it's caused by mutations in a gene called myosin 7A.

And if you look on the bottom right of this slide, this gives you an idea of what a patient with USH1B experiences. Typically, there's a constriction in the visual field as the rod photoreceptor cells in those patient retinas die off and they lose function. And the patient develops sort of this tunnel vision-like phenomenon.

So it's mutations in a gene called myosin 7A which cause Usher syndrome 1B. And what myosin 7A is is an actin-based molecular motor. Let's put this very simply. On the top, you can see sort of a description of what the protein looks like, but it has this head domain or this motor domain on the left, which binds to actin filaments in the cell. And then its tail domain, which is essentially really good at binding and grabbing onto other proteins in the cell. And we know that this protein is expressed in both the retina as well as the hair cells of the inner ear.

So within the inner ear, myosin 7A is very important in hair cells, as I mentioned. And if you look at the hair cell bundles, you can see that myosin 7A is responsible for grabbing onto actin filaments inside these stereo cilia, and then grabbing onto a bunch of other proteins in that area, which were referred to as the usherome, or the Usher interactome. But myosin 7A binds to a lot of other things and it helps stabilize the relationship between these hair cells, these stereo cilia and the hair cells.

And we know a lot more about what myosin 7A is doing in hair cells of the inner ear, actually, than we do about what it's doing in the photoreceptors.

What we do know or what's been reported, however, is that myosin 7A is expressed both within the retinal pigment epithelium as well as in the photoreceptors of our retina. And these are two cell types that are immediately next to each other at the back of the eye. We know that myosin 7A expresses in the RPE, and that it's involved in the transport of what we call melanosomes. So these dark circles here that you see in the RPE cell, in a normal retina, would migrate towards the photoreceptors into what we call the apical processes. In myosin 7A-deficient animals, that doesn't happen.

We also know that in photoreceptors, myosin 7A seems to be important for transporting different proteins from the inner segment of the cell into the outer segment. So again, myosin 7A seems to be important for functions within retinal pigment epithelium, as well as the photoreceptors. And we'll get into that in a little bit.

So why is hearing affected first? Well, it seems to be that myosin 7A is important for the development of hair cells in the inner ear. And what you can see on this slide are very high magnification images of hair cells within a normal mouse inner ear. And you can see that the hair cell bundles are very structured parts of the inner ear, whereas in a myosin 7A deficient mouse, called the shaker-1 mouse, you can see that those hair cell bundles are very disorganized, and in some cases, haven't developed at all. So this is the reason that USH1B patients are born profoundly deaf. It's because their hair cell bundles never developed properly.

But fortunately, myosin 7A doesn't seem to be required for the development of the photoreceptor cells because patients develop with photoreceptors quite normally. It's that their function is difficult to be maintained without myosin 7A. So my colleague, Sam Jacobson, at the University of Pennsylvania, has spent a number of years studying this disease. And he's looked extensively at the retinal structure of USH1B patients using a technique called optical coherence tomography, or OCT for short.

And what you can imagine OCT is, it's like an ultrasound of the eyeball that essentially gives you a cross-sectional view of a patient's retina. So up top, you can see an OCT scan through a normal patient's retina. And at the very center of that image is what we call the fovea, which is the cone-exclusive part of the retina. And what Dr. Jacobson has done is color-coded the photoreceptor cells and their outer segments in these OCT scans.

And then here are three different scans from USH1B patients. And you can see that each of these patients has exhibited a different level of photoreceptor structure loss. And patient 15 here, you can

see that all that really remains is photoreceptor structure within that cone-exclusive foveal region. And that makes a lot of sense if you consider what happens in the disease. Rod mediated vision is lost first. As I mentioned before, patients will develop sort of tunnel vision, and cone vision more slowly declines. It ranges from normal to severely reduced in the first four decades, but after that fourth decade becomes pretty abnormal.

One really important thing that Dr. Jacobson noted is that problems in this disease start in the photoreceptor cells. Even though I've provided you data that this protein is important in both photoreceptors as well as the RPE, the disease starts in the photoreceptor cells. So it's really important that when you develop a gene therapy for this form of Usher syndrome, that we target those really important photoreceptor cells.

So that brings us to that fact indeed, developing a gene therapy for this disease. One of the first things that's very important for developing a gene therapy is that you have a reliable animal model with which to test the gene therapy in. And in Usher syndrome, that has been a bit of a hurdle. So the mouse that everyone relied upon for many years was called the shaker-1 mouse. Very much like the patients, they have vestibular dysfunction, and actually, it presents a little bit more dramatically in the mice. If you look in their cage, they have circling behavior and head tossing.

But unlike the patient, these mice do not have any noticeable retinal degeneration and they have no loss of retinal function to be detected on an ERG. So this is not an ideal mouse model because it doesn't mimic the patient condition. So that led investigators, for a number of years, to try to find any phenotypes or anything that was wrong with the shaker-1 mouse mutant retina that could possibly be used as an outcome measure to evaluate whether or not our gene therapy would be effective.

So work that was done in David Williams' lab a few years back did indeed show that myosin 7A was really important for the migration of those melanosomes that we talked about before. It's somewhat hard to see, but if you look at the very top of this slide, this is a normal mouse RPE. And you can see that these little black dots, these little melanosomes, have migrated downward towards the photoreceptors in this normal mouse. Whereas in the myosin 7A deficient shaker-1 mouse, they all remain sort of stuck up in this RPE. And that's much more obvious if you look down at the bottom at these high magnification images of individual RPE cells with a normal cell on the left and a myosin 7A deficient cell on the right.

Dr. Williams also looked at rhodopsin transport through the connecting cilium of the photoreceptors. And what he saw was that in wild type mice that were immunogold labeled for rhodopsin-- so these

tiny little black dots you see are rhodopsin molecules in the outer segments of the photoreceptors-- he saw that there weren't many of those molecules within the connecting cilium. So they're obviously passing through this region, but they're doing so pretty quickly. And most of them are hanging out in the outer segments.

But in the myosin 7A deficient photoreceptors, he noticed a few more rhodopsin molecules were hanging out in the connecting cilium. It could be argued that this wasn't as dramatic a phenotype as what we saw in the RPE. But nevertheless, it did appear that rhodopsin transport may be somewhat impaired in these myosin 7A deficient mice.

So the shaker-1 mice were very useful for early studies of this disease, but they weren't that easy to deal with because they have a single point mutation that leads to their disease, which makes maintaining them as a colony very difficult for reasons that we won't get into today. But essentially, for many years, folks relied on their behavior to say that, yes, this is still a shaker-1 mouse, and yes, the daughter and the son of that shaker-1 mouse are also shaker mice because they still exhibit this behavior, which isn't the best way to maintain a mouse line. And from a genetic standpoint, they were a little bit more difficult to maintain. So we wanted to develop a traditional myosin 7A knockout mice that could be much more easily maintained from a genetic standpoint. And so we created that mouse model just a couple of years ago. It has a very simplified genotyping protocol, and we know that it's null for myosin 7A, which means there's no myosin 7A present.

And on this slide, I'm simply showing you proof that the myosin 7A knockout mouse does not express any myosin 7A protein in either its eyes, which you can see on the left, or in its ears, which you can see on the right. So this is an example of what we call a western blot. And we've essentially quantified protein expression levels in the myosin 7A knockout mouse, versus the heterozygous mice, versus the wild type mice. So you can see, there is no myosin 7A expression in the eyes or in the ears of those mice. So they are definitely a true knockout mouse model.

So in summary, we did a bunch of things to characterize this mouse model before we really started using it. And I'm just going to quickly go through those conclusions here. As I just showed you, the myosin 7A knockout mice do not express myosin 7A protein in either their eyes or in their ears. They are profoundly deaf. I didn't show you this data, but we did those types of analyses to prove that they were. They also have highly disorganized hair cells.

Interestingly, though, we found that their vestibular hair cells were fairly well-maintained. And we're not going to discuss this during today's talk, but maintenance of vestibular hair cells may provide us

an opportunity to correct that phenotype as well with gene therapy. Very much like the shaker-1 mouse, we did see that the retinal dysfunction in the myosin 7A knockout mice was very mild. So it's not going to be a useful outcome measure for gene therapy. There was also no appreciable loss of retinal structure over time. So the bottom line is that our newly-created traditional myosin 7A knockout mouse is very reminiscent of the shaker-1 mouse model.

But still, we wanted to ask, is there a better way to evaluate whether myosin 7A is really important for the transport of rhodopsin in photoreceptor cells of the mouse retina? And so we did that by crossing our novel myosin 7A knockout mouse with a mouse model that expresses rhodopsin fused to GFP. So put simply, the rhodopsin molecules in this mouse glow green. So we can cross them. We can breed them onto myosin 7A knockout mice and ask if the absence of myosin 7A affects the transport of that rhodopsin simply by looking at cross-sections of the retina and seeing where we see green. So really, kind of a simple concept.

So we compared that in mice that lacked myosin 7A, mice that carried one copy of myosin 7A, and mice that carried two copies of myosin 7A. And what we saw, to make a very long story short, is that myosin 7A didn't seem to play a role at all in the transport of rhodopsin through the connecting cilia of mouse photoreceptors. And in our positive control, which we knew would have problems, you definitely see trafficking problems and ultimately a loss of retinal structure. But myosin 7A doesn't seem to play a role in mouse photoreceptors that we can see.

And that brings about an interesting concept, which is why don't mouse models of USH1B have a phenotype like the patients? And there's some very nice work that Christine Petit has done to try and get at the answer to this question. And what it boils down to is that myosin 7A expression in mouse photoreceptors remains highly controversial. And what Christine proposes is that the reason for this is that mice lack a certain type of structure in their retina that primates have, or that humans have, that monkeys have. And that is called a calycal process. And in orange here, you can see in the middle of the slide, these calycal processes sort of form a girdle around the inner and outer segment of the photoreceptors.

And this is only a cartoon, but you can see in these real life electron micrographs that those calycal processes are very obvious. And when Christine Petit stained with an antibody directed against myosin 7A, she found that it localized to those calycal processes. So this is all in primates and monkeys. But when you look at mouse photoreceptors, they don't have those calycal processes. So maybe it's the structural difference between mouse and non-human primate or human that accounts for the fact that mice don't have this retinal dysfunction or degeneration like we see in the patients.

And this slide unfortunately has come up a little bit funny on my Mac computer, but what you can see on the bottom tells the story. What we did was we probed for the presence of myosin 7A in either mouse photoreceptors versus RPE, and compared that to what we saw in non-human primate photoreceptors versus RPE. And in mouse, we found that the vast majority of myosin 7A was expressed in the retinal pigment epithelium, or the RPE, whereas very little could be found in the photoreceptors. The exact opposite was true in the monkey retina. We saw that the vast majority of myosin 7A was present in the photoreceptors and not in the RPE. So this sort of gets to Christine Petit's data, that maybe the structural difference between monkeys and mice accounts for why there's not a phenotype in the mouse models. Because myosin 7A doesn't seem to be that important in the mouse retina.

So due to the difference in structure and expression, these mice are not going to be useful for better understanding USH1B disease mechanisms, or for testing outcome measures for proving that our gene therapy was effective. However, they're still going to be useful for evaluating the recombination efficiency of our vectors. In other words, can we express full length myosin 7A with our gene therapy vectors? And that can be done in a background that we know doesn't have any myosin 7A expression natively. And the added benefit, as I mentioned before, is that these mice are more easily genotyped or easy to maintain. So we can keep them as a valuable resource very easily.

So how will we deliver the myosin 7A gene to the retina? So gene therapy is a fairly simple concept when you boil it down. But all of us are made up of DNA, and there are subunits of our DNA called genes. And what we can do is take a gene of interest, like myosin 7A, and we can put it into a vector. Most often, that vector comes in the form of a virus. That virus will be directed to the cell of interest, in this case, the photoreceptors. It will then release its DNA, and then that DNA will go on to make the protein that we want it to produce. So in this case, the myosin 7A protein.

So a pretty simple concept, but one problem. The gold standard viral vector for delivering genes to patient cells is called AAV, or adeno-associated virus. And as you can see, it's relatively small compared to some of the other viral vectors that have been used in the field. And unfortunately, the myosin 7A gene is too big to fit inside the standard AAV vector. And for that reason, initial studies focused on using a larger vector Lentivirus to deliver this gene, because it could fit the entire gene.

And in fact, Dr. Williams's lab showed that a Lentivirus carrying the myosin 7A gene could correct that melanosome migration phenotype that we saw on the shaker-1 mice. It could also correct the opsin trafficking defect, although the results were spotty. But because of the success, this program was

picked by Oxford BioMedica a number of years ago. And their drug was called USHstat. And then that program was picked up by Sanofi. And it was shown to be very safe and well-tolerated. However, to date-- and it's been a number of years now-- there has been no evidence of biological activity, unfortunately.

Last year, Sanofi dropped the USHstat program. And just a few days ago, actually, Oxford BioMedica picked it back up again. So the jury's still out on this. Obviously, I'm vector agnostic. I hope that something works, whatever it is. But the entire time that this has been going on, we've been thinking about a plan B or an alternative approach to getting this job done.

And that involves adeno-associated virus, or AAV. And as I mentioned, this is the gold standard for delivering genes to patient retinas. The reason for that, it's non-pathogenic. It doesn't cause disease in human. It's relatively non-immunogenic. It can infect a cell and deliver its gene there, were that gene will be expressed over the lifetime of that living cell. And AAV also comes in a variety of flavors or different variants. So we can sort of select which variant is best suited for the cell type we're trying to target. And then most importantly, AAV was approved for the use in Luxturna, which is the very first FDA approved gene therapy to address this inherited retinal disease. So it's very clear that this viral vector has a solid track record.

So as I mentioned, the myosin 7A gene is too big to fit into a single AAV vector. So what we've done is develop dual AAV vector platforms, where essentially, we have split the myosin 7A gene in half. We've put one half into one capsid, and another half into another capsid, so two different vectors altogether. We then can infect those two capsids into a single cell. And via a region of overlap between those two sequences, recombination can occur and the full length gene is formed, which can go on to form full length myosin 7A.

So we have designed a couple of different dual AAV vector platforms that rely on slightly different mechanisms to promote recombination and ultimately make full length myosin 7A. We don't have time to get into the molecular biology behind that today, but suffice it to say, we're looking heavily at two different AAV vector platforms to get this job done.

So previous work for my lab, which was published back in 2014, looked at sort of the first generation of these dual AAV vectors using western blot. And you can see here in this gel, where we've probed for the presence of full length myosin 7A, that our hybrid dual vectors were driving the most efficient levels of full length protein when compared to simple overlap.

But what you'll also notice is that the hybrid vector system was producing a truncated protein, which

we believed was coming from that front half vector alone. The simple overlap platform was not doing that. The next thing we did was subretinally inject out the simple overlap vectors for the AP hybrid vectors into wild type mice. And we find that the simple overlap vectors were relatively well-tolerated, but the hybrid vectors led to a definitive loss of retinal structure and function.

And I should mention that when we initially did these experiments, we made the vector and we delivered it at the highest dose that we possibly could just to see what would happen. So this up here, 2E of the 10 total vector genomes delivered, is a very high amount of AAV to deliver to the retina.

So at this stage, we want to improve our myosin 7A dual vectors. And we want to ask the following questions. Can we use dual vectors to express myosin 7A at a level comparable to endogenous expression in mice? Is it going to cause any functional decreases? And if so, what's causing that functional decrease?

We also want to address this issue of that truncated protein that's coming from that front half vector, and we want to see if we can reduce or eliminate its production. And finally, we want to ask, are these dual vectors going to be well-tolerated in a clinically relevant model, in other words, a monkey?

So here's our experimental design. We're using dual vectors, but this time, in a much lower concentration than that that I showed you earlier that was leading to that retinal degeneration. We subretinally inject that vector into the myosin 7A knockout mice. And then at six weeks post-injection, we measure retinal function using a very typical assay called electroretinogram. And we measure retinal structure with OCT, like I showed you before.

So on this slide here, what you're looking at is a western blot comparing the levels of full length myosin 7A that were present in the retinas of mice injected with either AAV5 or AAV8 based vectors. And we looked at both the overlap and the hybrid vector platforms in the study. And those were compared to mice that were injected just with BSS alone, which is essentially like saline. And then we also included heterozygous mice in 7A mouse retinas, as well as wild type mouse retinas.

And what you can see is that the hybrid vector systems definitely drove the highest levels of myosin 7A expression, which you can see quantified in this graph to the right. And excitingly, we saw that the hybrid vector system was driving levels of myosin 7A that were equivalent to that seen in the heterozygous mice- in heterozygous mouse, excuse me. So we fully anticipate that these will be therapeutic levels of myosin 7A expression.

But remember, the hybrid system is also producing a truncated front half protein, which you can see

here. These are knockout mouse, or mice, rather, that were injected with the front half vectors only. So we have overlap or hybrid. And in this red box, you can see that truncated protein showing up. And when we inject those in the mice and then we look at the ERG response, as you can see, that there's actually a significant drop in retinal function in the mice that received that front half-- that front half vector.

So we thought some more about why we were seeing these functional decreases in mice that were injected with front half hybrid vector. And it got us into thinking about the structure of the protein. So our original hybrid vectors were split such that the front half vector encoded sequence corresponding to this neck and tail domain in the protein. And remember, I told you that this part, everything down from the motor domain, is really good at grabbing onto other things in the retina. And so we thought to ourselves it may be that we've accidentally injected something into the retina that's not the full length myosin 7A, but it's got just enough sequence to grab onto other things and cause problems but not actually do its job completely. So that's what we call a dominant gain of function.

And so it was suggested that we alter the split point by one of our colleagues who's a total expert in myosins. And he thought if we change the split point so that it was right here, that we wouldn't have any of that tail domain in our front half vector. And so we call these the hybrid V2 vectors.

We next tested them relative to the original hybrid vectors using the same clinically-relevant concentration, subretinally injected them into myosin 7A knockout mice, and evaluated them six weeks post-injection with ERG, OCT, and western blot. And the three main questions we were asking were, number one, will the hybrid V2 vectors with this altered split point still express full length myosin 7A at the same levels as the originals?

The second question was will the hybrid V2 front half vector produce truncated protein? Even though we've altered the slip point, we don't necessarily think that we're going to eliminate expression of a truncated protein. We just hope that it won't be toxic. And finally, will those hybrid V2 vectors cause any decreases in ERG amplitude?

So here are the results of that experiment. Here's the western blot on the left. And you can see that our hybrid V2 factors are producing just as much full length myosin 7A as the original hybrid vectors which are over here on the right. And that when you look at the quantification, both the original hybrids and the hybrid V2 vectors are actually producing myosin 7A to levels that, in this experiment, are actually a little bit higher than that seen in the heterozygous mice. So that's exciting news.

You will see, however, that the hybrid V2 vectors are still producing a truncated protein, which again, we didn't expect would go away. But the real question is, does it lead to loss of retinal function? And the exciting answer to that question is no. When we compared ERG results in mice that received original hybrid vectors versus the hybrid V2 vectors, you can see that there was no loss of retinal structure-- sorry, retinal function at this time point, and There, was also no loss of retinal structure, which we assess with OCT.

So we've shown that we can eliminate the toxicity caused by that front half truncated protein product. But the next question is, can we reduce or eliminate its formation altogether? So what we did, put simply, is we've looked at the sequence of that front half vector. And there were four sequences that essentially act as stop signs in that sequence. They don't necessarily make the protein formation stop, but they're almost like a yield sign. But there's the potential that these stop signs could further stabilize the production of the front half truncated protein, put simply.

So we altered those stop signs. We codon-modified them, such that they wouldn't be there either in stop sign or yield sign form, but the goal being to reduce formation of this truncated protein. And we found that codon modification or changing those stop signs in the hybrid 2-- in the hybrid V2 front half vectors actually did significantly reduce production of a truncated protein. So here's our V2 vectors, our original hybrid fronts, and then one of our front half vectors with three stop signs removed, and then one of our hybrid vectors with four stop signs removed. So this looked very promising, and we went on to pursue the hybrid V2 codon modified V2 front half construct for its ability to express full length myosin 7A when combined with the back half.

The next thing we did was we tested this new construct, this hybrid V2 codon modified V2 dual vector constructs in cells in vitro. So in other words, we took the plasmids that had those stop signs, combined them with the back half plasmids, and asked, could they also produce full length myosin 7A? And the answer to that question was definitely, yes. The plasmids with the stop signs were working just as well-- just as well to make full length myosin 7A as our original constructs.

The next obvious thing we wanted to address is whether or not these hybrid V2_CMv2 dual vectors-- in other words, the ones with the stop signs-- whether or not they caused functional or structural decreases in injected mice. And the answer to that question is no. You can see that in the ERG results at six weeks post-injection, the only functional losses that we saw were associated with the original hybrid vectors, whereas the hybrid V2 and the hybrid V2 with the stop signs are not causing any loss of retinal function. The same is true of retinal structure.

That was only a six weeks experiment, however. It's very important that we look at the long-term analysis of these vectors. And so we did a long-term study that went out for six months. And you can see here in these green circles, these are the ERG recordings of mice injected with the hybrid V2 vectors with the stop signs. And they look absolutely no different at all from the mice that were injected with BSS, or essentially saline alone. So we're very excited about these results, and we think that it means that the hybrid V2 vectors with stop signs hold a lot of promise.

The last question we wanted to address was, will these vectors be useful in non-human primate? And you've seen all the differences in structure between mouse and non-human primate retina. And I think I've driven the message home that in order for this gene therapy to be successful, our vectors must be designed to recapitulate the pattern of myosin 7A in a clinically-relevant species, in other words, one that actually has those calycal processes.

And so a couple of years back, I worked with a collaborator of mine, Paul Gamblin, at the University of Alabama at Birmingham, as well as Doug Witherspoon, who's a vitreoretinal surgeon. And we tested the simple overlap dual vectors in non-human primate. We subretinally injected them into adult macaques, just like we would in the mouse. And then we evaluated them with OCT and ERG. And what I'm showing you here is that, indeed, we did see expression of myosin 7A transcript in the subretinally injected monkeys, and that there was no apparent impact in the negative direction on either retinal function, which you can see here in the middle, or on retinal structure. And so put simply, this means that the dual vectors are well-tolerated in subretinally injected macaques. Now having said that, these were the simple overlaps. We only had two subjects with which to evaluate this. So our goal is obviously to move the hybrid vector system into the non-human primates and make sure that they are also well-tolerated.

So in conclusion, what we've shown is that all of these dual vectors are capable of expressing full length myosin 7A. And in terms of the hybrid vectors, we found that they produced myosin 7A to levels that were the same as what we see in a heterozygous mouse. So we fully anticipate that that will be therapeutic levels of expression. The front half vectors all produced a transcript or an RNA, but only the hybrid front half vectors produced a truncated protein. But we did see that that truncated protein from the original hybrid vectors did lead to a slight loss of retinal function in subretinally injected mice.

The back half vectors, put simply, don't do anything. They don't make an RNA or a protein. They cause no problems. We altered the split point of our hybrid dual vectors and our V2 vectors, and we found that we could eliminate the toxicity caused by the truncated front half protein. We also found

that we could codon-optimize that front half construct by modifying the stop signs, so to speak, to reduce the production of that truncated protein. And we hope once we get into our in vivo experiments, that we'll show that the production of that truncated protein may be eliminated altogether. And finally, we show that the dual AAV5 vectors do produce full length myosin 7A in subretinally injected primates and are well-tolerated.

So finally, I'm very excited to announce that we have recently created a new company called Atsena Therapeutics. We have a great team. We have some great major investors, the Foundation Fighting Blindness and Hatteras Venture Partners. The fact that the Foundation Fighting Blindness has backed us is-- really lends a lot of confidence to the fact that our focus is going to remain on helping these patients. And one of Atsena's main programs is going to be developing a dual AAV vector-based treatment for USH1B. So we're very excited to have the financial means to continue this research, to test these dual vectors in non-human primate, and hopefully bring a dual AAV-based vector into patients in phase 1/2 clinical trials in the near future.

So with that, I want to thank everybody in my group that's been involved in the USH1B studies and every other crazy project we do in the lab. But most importantly, I want to thank Katelyn Calabro, who has been one of the most dedicated members of my lab that I've ever had the pleasure to work with. And her baby has been this USH1B project. So a huge thank you to Kate and also Sanford Boye, who is my colleague and my husband, if you look at his last name. He's also obviously been integral in this pursuit, and Sean Crosson, who is another postdoc of mine who has also worked a lot with Kate on this project. You can't do anything without a good team, and that, I definitely have. And then finally, I'd like to thank the Foundation Fighting Blindness for providing funding for this very important project. So with that, I thank you for your time, and you're welcome to reach out to me with any questions you might have. Thank you so much.