Usher Syndrome Coalition | Gene Therapy for Usher Syndrome Type 1F: Engineering mini-PCDH15s for Viral Delivery, David Corey, PhD

It's a great pleasure to be asked to speak at the Usher Connections Conference. And today I'd like to tell you about work that my laboratory and our collaborators have done on trying to develop a gene therapy for Usher syndrome, specifically Usher type 1F. As all of you know, Usher syndrome type 1 is the most severe of the Usher syndromes. And it can be caused by mutations in any of five different genes. Usher 1F in particular is caused by a variant or a mutation in the gene encoding the protocadherin-15 protein.

It's a fairly rare disorder in the United States. Maybe 50 children a year are born with Usher 1F, a total of perhaps 3,000 patients in the United States, and maybe somewhere around 10,000 worldwide. There are a number of different variants or mutations in the gene encoding protocadherin-15. Some of them marked in red cause both deafness and blindness, the full Usher syndrome. And some of them cause deafness only.

And it's interesting that some of these mutations that only cause deafness don't cause blindness also. And it makes us think that there may be greater demands on the protocadherin-15 protein in the ear than in the eye. And this gives us hope that a therapy that restores function in the ear may also be promising in the eye.

We've studied the auditory system for many years. And let me tell you a little bit about what we've learned about the function of protocadherin-15 in the auditory system. As you know, the external ear collect sounds and funnels it through the ear canal to where the sound makes the tympanic membrane, the eardrum, vibrate. And this vibration is carried through the middle ear to the inner ear.

The inner ear actually subserves two different senses. These organs give us a sense of balance. And this organ here, the cochlea, subserves the sense of hearing. Within the cochlea, there is a narrow ribbon of sensory cells, the receptor cells, that convert the soundwave into a neural impulse. And we can get a sense of what those look like if we cut a cross-section through just one turn of the cochlea.

And so we'll zoom in. And you can see here in this one cross-section, the sensory cells themselves are just these that are located in this narrow ribbon. The sound information is carried from the sensory cells to the brain by fibers of the auditory nerve. And the cell bodies of the auditory nerve fibers are right here in this region called the spiral ganglion.

If the receptor cells are not functioning, a cochlear implant can be delivered which gives mild shocks

directly to the neurons of the auditory nerve and therefore can carry auditory information to the brain. But we'd much prefer, of course, to be able to restore function in the receptor cells themselves. So let's look a little bit further at the receptor cells.

Here you can see in a scanning electron micrograph the receptor cells I've colored in blue. And there are actually two types. There are outer hair cells that are called hair cells because each one of them has this tuft of cilia-like processes coming from the top surface, perhaps a hundred of these cilia on each cell. And then there are inner hair cells that have a very similar structure, perhaps fewer of the cilia.

If we zoom in to look at the cilia on an inner hair cell, you can see perhaps that there is a fine filament that extends from the tip of each cilium to the side of the taller adjacent cilium here, here, and here. And I'll just sort of draw in these filaments. What's interesting is that the filaments are made of two different proteins. One of them is cadherin-23. And we know that mutations in cadherin-23 also produce Usher syndrome type 1. And the other is the protocadherin-15 responsible for Usher syndrome type 1F.

If we look in more detail at what this filament does, we can see, once again, the cilia in this case on a hair cell from a balance organ. And if you look with the electron microscope at the tip of one cilium, you can see this fine filament, which is called the tip link, extending from the tip of one cilium, again, to the side of the next taller one. And this little filament schematized here is made of two strands of cadherin-23 and two strands of protocadherin-15. The protocadherin-15 is attached at the lower end to a protein that's called an ion channel. And when tension on the ion channel is increased, the channel can open, allowing electric current to flow into the cell and generating a signal that the nervous system can understand.

Sound makes the cilia vibrate back and forth at sometimes thousands of times a second. And you can see that each time the cilia move towards the right, the tips of the cilia move further apart, schematized here. And that would increase the tension on the tip link. And that would increase the tension pulling this channel open. Of course, if someone isn't making protocadherin-15, then the tension cannot be applied to the channel, and there's no way of sensing the vibration of sound.

Protocadherin-15 is also used in the receptor cells of the eye, the photoreceptors, specifically, of the retina. So if we kind of look at a small section of the retina, there are roughly five different layers of different cell types. But these cells towards the outside of the retina, the photoreceptors, are the ones that absorb light and generate an electrical signal. Photoreceptors are characterized by having an

inner segment and an outer segment. The outer segment is the light sensitive part of it.

And it's interesting that in human photoreceptors, similar to these from a type of monkey, there is this group of sort of finger-like processes-- what are called calyceal processes that extend from the inner segment out around the outer segment. And they may serve to stabilize the outer segment. And they also seem to be involved in the synthesis of the outer segment. So protocadherin-15 is actually found associated with these calyceal processes and seems to be involved in the stabilization of the outer segment.

How could we do something to treat the genetic defect that causes Usher 1F? In a number of cases, a simple viral vector, adeno-associated virus, can be used to develop therapeutic genes. And the basic way that this works in many diseases is as follows. This is sort of a package that can hold DNA. And if we imagine a hereditary disorder that's recessive, then two parents may each carry a mutation.

But if they have a normal copy of the gene, then they don't have the disease. But if a child is unlucky enough to inherit both of the defective copies of the gene, then they would have the disease. So what we'd like to do with the AAV vector is to carry a normal copy of the gene into the cell. That DNA can be used by the cell to produce new protein and protein that would function properly. And we hope that that would restore the normal function of the cell and treat the disease.

The interesting problem with Usher 1F is that the DNA that encodes protocadherin-15 is very long, about 6,000 letters of DNA. And adeno-associated virus can only hold about 4,500 letters of DNA. So the challenge for our lab has been to try to figure out a way of getting the DNA into an AAV.

A strategy that we've taken is to try to cut out parts of the DNA that may be unnecessary. So you could imagine that if we could just snip out some part of it and then join the two bits together, then that bit of DNA might be small enough to fit into an AAV. And then we could carry that into the hair cells and into the photoreceptors in the Usher 1F patient, hopefully to restore the normal function.

So how would we remove unnecessary segments? And what we've tried to do then, is to make a miniature version or a mini protocadherin-15. Well, we're very fortunate in that work over the last 10 years, mostly by Marcos Sotomayor-- originally in my lab, and now in his own laboratory at Ohio State-- has been able to solve the entire X-ray crystal structure of this part of protocadherin-15. The position of every one of nearly 20,000 atoms has been mapped. And so we know exactly what it looks like at an atomic level.

We know that at this end, it attaches to the cadherin-23. So that's very important. We know that at

this end, it attaches to the ion channel. And that's important. But we thought maybe we could snip out some of the segments in the middle, segments that are a bit like links in a chain, and just rejoin the links to make something shorter. It's important, as I said, that we know the exact atomic structure because we can look at the position of individual atoms where we would join the links together, and we can engineer a protein by modifying the DNA that would have the links in the exact position to form a strong connection.

So we need to do some tests then on the mini protocadherins that we engineered. The first question is, do these behave properly in a test tube? For instance, do they bind to cadherin-23? And I won't go into detail, but it's clear that they do bind cadherin-23. And they perform normally in other tests that we've run.

The second is, is there a good animal model? Is there an animal that seems to show the symptoms of Usher 1F? So what we've done for that is to look at mice or zebrafish or primates to find one that would be an appropriate model. And we began by making a line of mice that lack protocadherin-15 only in the inner ear. So we can ask, do they have the deafness that's characteristic of Usher 1F?

So here is the hearing of a normal mouse in the dashed line. And the way that we measure the hearing is to play sounds to the mouse, and then put a fine wire on the back of the head that picks up the tiny, tiny electrical signals that are generated in the brain when a mouse hears. And we can ask, what is the quietest sound measured here in decibels that can elicit a barely detectable electrical signal? In a normal mouse, from frequencies between about four kilohertz and 32 kilohertz requires sound of maybe 30 or 40 decibels to elicit an electrical response.

But the knockout mouse that we made gives no electrical response even at 85 decibels, which is the loudest sound that we gave. So this is a deaf mouse. And it seems to be a good model for testing restoration of hearing. So yes, we have a good animal model, at least for the hearing at this point. Now a question is, how can we deliver the DNA to the inner ear?

The way that we would do this is to, again, take the DNA that encodes protocadherin-15. We add it to DNA that will put it into the AAV vector. And then we can put that DNA inside the AAV, inject billions of copies of the vector into the inner ear of a mouse.

And this is, again, a mouse that has the protocadherin-15 knocked out and would normally be deaf. We can then let the mouse grow up and see if we've been able to restore the hearing. And we would do that again by playing sounds to the mouse and recording the tiny signals that come from the brain.

So how does this work? Here's the first version we tried of mini protocadherin-15 version A. Once again, here's the hearing of a normal mouse that has not had the protocadherin-15 deleted. And it can hear quite well.

Here is the hearing of the knockout mouse that's completely untreated. And what we'd like to do is to bring the hearing of the knockout mouse down towards the hearing of a normal mouse. And when we tried version A, we found that it didn't help at all. This version is completely non-functional. It doesn't rescue the hearing in this mouse.

We tried another version, version D. And we were excited to see that when we injected the AAV containing mini protocadherin-15D, then we were able to restore the hearing. Now sounds as loud as about 60 decibels, which is the level of a normal conversation, could elicit an electrical response.

And so this mouse could hear, although it took a louder sounds to produce the hearing. However, if we injected that same virus into a normal mouse, we found that it actually damaged the hearing. Instead of being sensitive down to about 30 decibels, it required nearly 70 decibels to elicit a sound. So that's not good. Version D is not a very promising one.

We then tried version E. And we're quite excited that this one seemed to work pretty well. If you inject the version E DNA into the knockout mouse, then most of the hearing is restored. Now sounds as quiet as 40 decibels can elicit a response. And that's nearly as good as the normal control mouse.

And if we injected the vector into a normal mouse, it didn't make the hearing any worse. So we're really quite excited about the possibility that this particular mini protocadherin could restore the hearing in larger animals and perhaps in human. And we'll be pursuing this and other versions in testing the restoration of hearing.

Well, an important part of protocadherin-15 deficit that is of Usher 1F is the loss of vision over 10 or 20 years. And we need to find a good animal model for the loss of vision. And this has been more complicated. For the mutant mice that lack protocadherin-15, others have tested their vision. And they find that the electrical response from the eye is nearly the same in normal mice and in mice that lack the protocadherin-15.

You can see that these electrical traces are practically the same. And they're practically the same between the normal mouse and the mutant mouse, both in dim light and in bright. When we look at the retinas of the knockout mouse, and we really don't see much change between the knockout and

the control-- that is, the photoreceptors don't regenerate in these mice.

A reason for that is that these calyceal processes where we said the protocadherin-15 was located are actually missing in the mouse. Monkeys and humans have them but mice do not. And so if mice don't even have the calyceal processes, they may not need protocadherin-15 very much in order to maintain the function of the photoreceptors. So we need a better model.

It turns out that this tiny fish only about two inches long called a zebrafish actually has photoreceptors that are closer to those in human than the mouse photoreceptors are. They have the same Usher genes. And mutations in Usher genes in the zebrafish produce death of the retinal cells. Now we're very fortunate that Monte Westerfield and Jen Phillips at the University of Oregon have created a zebrafish model of Usher 1F by making a mutation in the protocadherin-15 gene in the zebrafish. And Monte and Jen sent us these fish so that we can study the vision in zebrafish that are lacking in protocadherin-15.

We first used electron microscopy to look at the photoreceptors in the larvae of a zebrafish. This is a fish that's only seven days old. But it has normal photoreceptors. And you can see that the photoreceptors in the zebrafish here at a higher magnification have the calyceal processes. And these may be important for the function of the photoreceptors.

In fact, when we looked at the mutant zebrafish, we could see first that the calyceal processes were disrupted-- that is, in the absence of protocadherin-15, the photoreceptors weren't developing very well. And in general, the photoreceptors were more disorganized in their appearance. Does that mean also that the photoreceptors don't function properly?

And here, we've begun to test the function of the photoreceptors in the zebrafish. We have two ways of doing this. Here is a seven-day-old zebrafish. It doesn't look quite like the adult, but it functions pretty well. And if we put an electrode on the surface of the eye and flash a light, then we can measure the electrical response from the light and see if that's compromised.

Another way of testing the function of the photoreceptors in the zebrafish is to put the fish in a rotating drum that has stripes on it. The fish can see the stripes and it tends to track the stripes, so that if we start the drum spinning, you can see that the eyes move to follow the moving stripes, and then turn back when they have gone as far as they can go. And we can plot the movement of the eyes to get a sense of whether the fish can actually see the movement.

So the experiments will be to inject the DNA for the mini protocadherin-15's into the eggs of the

zebrafish, shortly after they've been fertilized, and to see whether we can restore the visual function in the zebrafish. And unfortunately, these experiments were put on hold by the coronavirus. But we're just now getting back to them. And we're very optimistic about being able to potentially rescue the function of the vision in this model.

So for hearing, we've been very pleased that version E of the mini protocadherin-15 preserves most of the hearing in mice without producing toxin. We're getting started on testing these mini protocadherin-15's in the zebrafish. I should point out that this work is a collaboration among seven laboratories with more than 20 people working on it, each bringing complementary skills. And we think we've got a great team and are optimistic about what we may be able to do to bring a therapy to Usher 1F.