Hello. This is Jennifer Lentz from LSU Health-New Orleans in Louisiana. Thank you so much to the Usher Syndrome Coalition for the invitation to talk about our research in Usher Syndrome Type 1C. Today, I'm going to start with a very brief review of Usher syndrome for those of you who are new to Usher. Then I'll talk about the research in our lab, our research mission, and the tools we use, and the development of two new therapies using antisense oligonucleotides for Acadian USH1C and gene replacement therapy for all USH1C.

Usher Syndrome, abbreviated USH, or simply US, is the leading genetic cause of concurrent hearing and vision impairment. Some individuals also have imbalance. The hallmark of Usher is that patients have both hearing and vision impairments. It's estimated that approximately one in 20,000 individuals in the world have Usher. Currently, there are three clinical types and 11 subtypes that are associated with Usher.

We use the numbers Type 1, Type 2, and Type 3 to describe the clinical type, which is based on the severity of symptoms. We also use letters to denote the subtype, which is based on which gene has a mutation. Today, there are six USH1 subtypes that are known, 1B, 1C, 1D, 1F, 1G, and 1J.

There are three USH2 subtypes, 2A, 2C, and 2D, and two USH3 subtypes, 3A and 3B. Each of these subtypes is caused by mutations in different genes, which are listed in parentheses next to the subtype at the bottom of the slide. A diagnosis of Usher syndrome is established with clinical features based on the severity of sensorineural hearing impairment; the presence of vestibular areflexia, which causes imbalance; and the age of onset of retinitis pigmentosa, which is a progressive visual loss that begins with night blindness.

Individuals with Type 1 typically have severe to profound hearing impairment at birth, balance difficulties, and retinitis pigmentosa, beginning in early adolescence, usually between the ages of seven and 12 years. Individuals with Type 2 typically have mild to severe hearing impairment at birth, usually mild in the low frequencies and severe in the high frequencies, and RP beginning in late adolescence to early adulthood, usually between 18 and 21 years of age. USH2 patients typically report having good balance.

Individuals with USH3 have later onset of hearing impairment and retinitis pigmentosa, usually later in adulthood. And some individuals also have balance difficulties. Most individuals with Usher have symptoms that suggest one of these types. However, many do not and have an atypical Usher, with symptoms from several types.

Individuals with both a sensorineural hearing impairment and RP without symptoms in other organs are generally given a diagnosis of Usher syndrome. Genetic testing then confirms the diagnosis and determines the specific genetic mutation that is the cause of the patient's hearing and visual symptoms. A multigene testing panel is most commonly used.

Today, there is a multigene panel specifically for Usher syndrome. But because the symptoms don't show up at the same time, inherited retinal dystrophy or hereditary hearing loss panels are often used because they contain the Usher genes, which are listed below.

Today, treatments for Usher syndrome include, for Usher 1, cochlear implants or sign language are used for the hearing impairment. Occupational and physical therapy can be used to manage balance difficulties and low vision aids for their vision impairments. For USH2 and USH3 patients, hearing aids, cochlear implants and low vision aids are used.

There are several new therapies under investigation in the clinic that are being tested in Usher patients. UshStat is a retinal gene replacement of the myosin VIIa gene for USH1B patients. This trial began in 2014 and has completed the dose escalation phase.

However, it is currently not recruiting. For more information, you can go to clinicaltrials.gov. The clinical trial identifier is listed here.

QR-421a is a retinal antisense oligonucleotide treatment for USH2A patients with USH2A exon 13 mutations. ProQR Therapeutics is sponsoring this trial. Three-month interim findings, which were reported in March of this year, showed the ASO is safe and well-tolerated in eight USH2A. And two patients also showed improvements in retinal sensitivity, retinal structure, and visual fields.

For more information about the interim findings or the trial, you can go to the ProQR website or clinicaltrials.gov. And I've listed the identifier here. This trial is currently recruiting.

CL-17-01 is a retinal antioxidant treatment for retinitis pigmentosa with Usher syndrome. This trial is sponsored by Nacuity Pharmaceuticals and is being held in Australia. It's not yet recruiting yet. But for more information, you can visit Nacuity's website or clinicaltrials.gov.

NPI-001 is also an antioxidant given orally to patients with RP that includes all USH types. They have completed a 30-patient study that showed it was well-tolerated and improvements in retinal sensitivity. They're currently conducting an extension study. For more information about this drug, you can visit Nacuity's website.

In addition to therapies under investigation in the clinic, there are several research labs developing new therapies for USH1C. At the NIH National Eye Institute, Dr. Tiansen Li and Dr. Anand Swaroop have developed USH1C Retinal organoid models from several USH1C patients' fibroblast skin cells. Currently, they're screening known molecules and other drug candidates. This work is sponsored by Usher 2020 Foundation.

In Johannesburg Gutenberg, University of Mainz, and LMU Munich, Dr. Uwe Wolfrum and Dr. Nikolai Klymiuk have developed a transgenic USH1C pig model with hearing, balance, and visual deficits. Currently, they're characterizing the pig model, studying mechanisms of USH1C disease and creating a breeding herd to test new therapies. This work is sponsored also by Usher 2020 Foundation and FAUN Foundation.

At Oregon Health and Science University, Dr. John Brigande is developing an USH1C non-human primate model and genetic therapy approaches for USH1C. This work is sponsored by NIDCD at NIH. Odylia Therapeutics was founded by Mass Eye and Ear and Usher 2020 Foundation as a nonprofit company to bring rare retinal disease therapies to the clinic. In collaboration with doctors Uwe Wolfrum, Kerstin Nagel-Wolfrum, Nikolai Klymiuk, Pigmod, and other experts, they're developing a comprehensive drug development plan to bring USH1C gene replacement therapy to clinical trials. Currently, they're testing AAV gene therapy in the USH1C pig model. This work is sponsored by Usher 2020 and FAUN Foundations.

So now I'd like to shift gears and talk about the research in my laboratory, which focuses on USH1C. This type is more common in our community because there is a founder mutation in the Acadian populations of Louisiana and Canada. Our research mission is to understand disease mechanisms or how genetic changes cause hearing, balance, and vision impairments, and to develop new therapies for the treatment of hearing impairment, imbalance, and visual loss associated with Usher syndrome.

The Usher subtype, USH1C, is caused by mutations in the USH1C gene. The gene is on chromosome 11 and contains 27 exons that are used to encode three families of harmonin proteins that are called Harmonin-a, Harmonin-b, and Harmonin-c. The Harmonin proteins are found in the ear and eye.

This is a diagram of the USH1C gene with 27 exons, which are the colored numbered boxes. And, today, 47 different mutations are known to cause USH1C. You can see here in exon 3, the 216 G to A mutation is circled in blue. This is the founder mutation in Acadian populations of Louisiana and Canada.

I've color-coded the mutations based on the type of mutations. Those in red are nonsense mutations that truncate the protein at that position. Those in orange are missense mutations that cause a single amino acid at that position to be changed.

Those in blue cause defects in splicing or the processing of the messenger RNA that is used to make the protein. And those in green are a deletion or insertion of DNA base pairs. Although these mutations work in different ways, they all result in essentially no functional Harmonin protein in the ear and the eye, which causes USH1C.

The 216A mutation is a splicing mutation. Splicing is the process by which introns are removed to produce a mature messenger RNA that only contains exons used to make a protein. You see in the diagram at the bottom of the slide an example of correct splicing, where the introns between exons 1 and 2 have been cut out, and these exons stitched together.

The splicing proteins-- here represented by scissors-- are now cutting out exon 3. This continues until all the introns are removed and only exons remain, which are the code to make the protein. The 216A splicing mutation is a founder mutation in the Acadian population. The G to A change at position 216 causes aberrant splicing.

You can see in the diagram at the bottom on the right that the 216A position tells the splicing proteins to cut at the wrong place. This causes the end of exon 3 to be cut out with the intron, which results in a truncated messenger RNA and protein and essentially no functional Harmonin protein produced in the eye and the ear. To understand how this works and develop therapies, we put the 216A mutation into the mouse genome to create a knock-in mouse model of Acadian USH1C.

The mice, which have the same mutation as patients, also have similar symptoms, severe to profound hearing loss; with abnormal auditory brain stem responses, or ABRs; balance problems with circling and head tossing behavior; and mild vision loss with reduced electroretinagrams and slow photoreceptor cell loss. We're using this model to test new therapies for USH1C. We designed an antisense oligonucleotide similar to QR-421a developed by ProQR, that is currently being tested in USH2A patients, except that ours targets the 216A mutation in the USH1C pre-spliced RNA to correct splicing.

ASOs are short pieces of nucleic acids that bind to their target. The 216A targeted ASO is designed to bind the 216A mutation, which blocks the splicing proteins from cutting at the wrong place and forces correct splicing. We have tested the ASOs in the USH1C mice to see if the concept works.

ASOs injected systemically restored hearing thresholds in USH1C mice. The chart below shows the hearing results of USH1C mice treated with ASOs. The black line is from normal hearing mice.

And you can see that they can hear very low intensity sounds, around 20 to 25 decibels, which is about like a whisper. In contrast, the USH1C mice, which is the red line, cannot hear sounds below 90 to 100 decibels. They are severely to profoundly deaf.

The blue lines are from USH1C mice treated with the ASO at post-natal day 1, or one day after birth. At one month of age, they can hear low-frequency sounds at levels similar to normal hearing mice. And this single treatment lasts for at least six months, which is the dashed blue line. There's also some improvements to the ABRs.

ASO treatment also restores balance behavior in the USH1C mice. In this video of the behavior of the mice in their cage, you can see the two mice on the top are normal mice with normal behavior. The mouse on the bottom left is an USH1C mouse that was given a placebo, and this mouse has lots of circling behavior that shows his balance is off. The mouse on the bottom right is also an USH1C mouse that was treated with the 216 ASO and is behaving more like the normal mice. We don't yet know the optimal time for treatment and are treating older mice to see if the ASO corrects their balance in adulthood.

We have also treated the USH1C mice to see if the ASO corrects vision. The graph below shows the results of electroretinagram analyses in normal mice, represented by the black line; USH1C mice that are untreated, represented by the red line; and USH1C mice treated with ASOs, which are the blue and green lines. You can see that the normal mice have a higher visual response to bright light compared to the USH1C mice.

ASOs injected one time directly into the eye by intravitreal injection restores visual function in USH1C mice for three months. By six months, the drug has worn off. But if we give additional treatments, one treatment every three months, we can maintain good visual function similar to that of the normal mice for at least one year. This is the line represented in green.

Our next steps are to continue to develop this approach using ASOs as a treatment for visual loss in USH1C mice. This is a diagram of our ASO drug development plan. We have completed the ASO target identification and validation, meaning we have identified targeting the 216A mutation with an ASO and showed that it works in USH1C mice. Our current NIH-funded grant is to identify a lead ASO drug candidate and identify patients and determine which test in the clinic would be most useful at measuring whether the ASO was working.

The next steps in drug development include proving a drug is safe; manufacturing it for humans; and applying to the FDA for a clinical trial. To identify a lead ASO drug candidate, we are optimizing the ASO. We're making small changes to the drug, testing them in the USH1C mice, and comparing the results to our current best-performing ASO, which improves vision by 20% to 40%, for three months. We're asking whether we can increase the ASO activity to be better than 20% to 40% or increase the duration of effect to work longer than three months.

We have designed 100 to 200 ASOs with slight modifications in sequence and chemistry. Currently, we have completed approximately 65% of the testing and are waiting for long-term studies for some of them. Once the testing is complete and we've identified an ASO with the highest activity or the longest effect, the next step is to prove it's safe.

In Aim 2 of our grant, we are identifying USH1C patients through a retrospective natural history study. For this study, we are enrolling all Usher patients that are Louisiana residents and all USH1C patients from around the world. Currently, we have 103 participants enrolled, 75 from Louisiana, 12 from Canada, and 16 from around the world.

Approximately 50% are males, and our participants range in age from 18 months to 93 years. 72 of 103 have genetic confirmation. We're working with the remainder to provide genetic testing.

Of the 103, 90 have USH1. And 65 of those have USH1C. One patient has USH1B, and one has USH1D. Eight have USH2.

And of those with genetic testing, both have USH2A. Only three have USH3. And three with genetic testing have USH3A. One patient has atypical USH, and one patient does not have Usher syndrome.

To determine the clinical outcomes measures that could be used to guide a future clinical trial, we're conducting a prospective natural history study. We're enrolling USH1C patients between the ages of 12 and 65 years for four clinic visits, one visit every six months for two years. Participants see our retinal specialists, Dr. Maria Reinoso at LSU Health New Orleans; Dr. Robert Koenekoop at MUHC in Montreal, Canada; or Dr. Wadih Zein at the National Eye Institute. Currently, we have eight participants enrolled that are waiting for their first clinic visit, which had to be rescheduled because of COVID restrictions. Fortunately, the restrictions have been lifted, and we are now working with the patients to schedule their clinic visits.

Now I'd like to talk about our efforts to develop a gene replacement therapy for USH1C. We know that many USH1C patients would not benefit from our ASO targeting the 216A mutation because it's a very rare mutation. There are many other mutations that cause USH1C, and so we're also developing a gene replacement therapy that would benefit all USH1C patients.

The idea is to deliver a normal copy of the USH1C gene to restore functional harmonin protein. We're working with AAV vectors to deliver the gene. These are replication deficient, which means they cannot reproduce without a helper virus present in the same cell. Also replication deficient AAV vectors are not known to cause disease in humans.

Our laboratory collaborated with Dr. Gwen Geleoc at Harvard to test gene replacement therapy for hearing and balance. Gwen's lab designed and tested the AAV USH1C in our USH1C mice. Her group showed that AAV USH1C b type restored hearing and balance in USH1C mice.

The graph at the bottom left shows significantly improved ABRs of USH1C mice treated with AAV-b, which is the green line. Hearing thresholds were significantly lower than USH1C mice that were not treated, which in this graph is the blue line. Also the AAV therapy corrected balance behavior.

Interestingly, AAVs expressing USH1C-a or USH1C-b or a combination of both improved balance behavior. The pictures above the graph on the right show the foot pattern of the mice. The three pictures in the bottom panel are from USH1C AAV-treated mice, and their pattern looks more like the upper left picture from the normal mice with no circling. The picture on the top panel in the right is from untreated USH1C mice that shows lots of circling behavior, indicating balance difficulties.

We're now working to redesign the AAV therapy for the eye, as a treatment for visual loss in the mice. The gene therapy is injected directly into the eye for subretinal injection. In the mice, we use a fluorescent reporter so that we can see that the injection procedure went well.

Four weeks after treatment, we can see green fluorescence in the fundus pictures, as well as in histology of the retina. The gel pictures on the right show thick black bands of full-length USH1C in the AAV-treated eye but not in the untreated eye of USH1C mice. We're currently aging the mice to see if the therapy corrects vision.

In summary, I've told you about two different therapies we're developing for USH1C. Our results show that ASO therapy targeting the 216A mutation restores hearing, balance, and vision in USH1C mice. Currently, we're optimizing this ASO drug to improve its activity or duration of effect as a treatment for visual loss in Acadian USH1C.

We're also developing a gene replacement therapy for all USH1C, in collaboration with Gwen Geleoc. Her group has shown that gene replacement therapy restores hearing and balance in USH1C mice. We have redesigned the gene therapy for the eye and are currently testing it as a therapy for visual loss in the USH1C mice. We're also conducting several natural history studies for patients, a retrospective natural history study to improve our understanding of the natural clinical history of Usher syndrome in Louisiana; a prospective natural history study of visual loss in USH1C; as well as a prospective natural history study of imbalance in USH1C.

I haven't had the time to tell you about this study, but it's very similar to the natural history study for vision loss, except these patients see our balance specialists at LSU. You can contact me for more information about participating. Finally, I would like to acknowledge all of those in my lab, my collaborators, and my funding sources listed here that make this research possible. Thank you.