OPEN

Retinal Gene Therapy for Usher Syndrome: Current Developments, Challenges, and Perspectives

Astra Dinculescu, PhD
Brian A. Link, PhD
David A. Saperstein, MD

Introduction

Usher syndrome (USH) represents a group of genetically heterogenous autosomal recessive disorders, characterized by combined vision and sensorineural hearing loss, and in some cases vestibular dysfunction.[1–3] It primarily affects the light-sensitive photoreceptor cells in the retina and the auditory hair cells in the cochlea. Vision loss in all cases is progressive, and manifests as retinitis pigmentosa (RP), characterized by the gradual degeneration of the photoreceptor cells.[4] Peripheral rod function is lost first, leading to night blindness and constricted visual fields, followed by the death of cones and severe visual impairment. There are currently no drugs or biological therapies proven to be effective in treating USH syndrome. Cochlear implantation, which bypasses the damaged hair cells and stimulates the primary auditory neurons directly, is an effective approach to alleviate the hearing impairment in patients. However, there is no remedy for the progressive loss of the photoreceptor neurons in the retina, and thus a critical unmet need exists to develop therapeutic strategies to prevent blindness in USH syndrome. The generation of animal models that faithfully mimic the human USH disorder, the timing of gene therapy interventions, the identification of correct target cells and patient selection are key factors in successfully reaching this goal.

USH syndrome was named after Dr Charles Howard Usher, a Scottish ophthalmologist who described 69 patients with the disease in 1914.[5] As is commonly the case in medicine, the disease was first described much earlier (1858) in brothers suffering from blindness and deafness by von Gräfe[6] and was further characterized by his student,

INTERNATIONAL OPHTHALMOLOGY CLINICS
Volume 61, Number 4, 109–124

Liebreich,[7] 3 years later in a larger population of patients. From its discovery until recently, USH syndrome and its subgroups were delineated by clinical characteristics.[8] Now the disease is recognized by symptomology and classified by genotyping. To date, there are at least 10 causative genes that are associated with USH syndrome, summarized in previous reviews.[1,2,9] In general, based on its diverse clinical symptoms, particularly the onset and severity of the sensorineural hearing impairment and the presence of vestibular dysfunction, USH syndrome is grouped into 3 clinical subtypes. Type I disease is associated with severe-to-profound prelingual hearing loss, vestibular abnormalities and prepubescent onset of RP symptoms.[10,11] USH1 results from mutations in *MYO7A* (USH1B), *HARMONIN* (USH1C), *CDH23* (USH1D), *PCDH15* (USH1F), *SANS* (USH1G), and *CIB2* (USH1J). Patients with type II disease display moderate-to-severe congenital hearing loss without vestibular dysfunction, and account for $>50\%$ of all USH cases. Their RP symptoms generally begin in the second decade.[12] USH2 is caused by mutations in *USH2A* (USH2A), *ADGRV1* (USH2C), and *WHRN* (USH2D). Patients with type III disease caused by mutations in *CLRN1* (*USH3A*) gene have postlingual progressive hearing loss, variable vestibular dysfunction, and variable onset of RP that rapidly progresses to legal blindness by the fourth decade of life.[13–17] Mutations in the HARS gene (Histidyl-tRNA synthetase) are associated with the ultra-rare form USH3B.[18,19] Other genes have been described in cases of atypical USH, including: *CEP250* and *CEP78*, encoding members of the CEP family of centrosome-associated proteins; *ESPN*, encoding the F-actin cross-linker espin; *ARSG*, encoding the arylsulfatase G enzyme.[20]

■ Gene Therapy for USH Syndrome: Current Challenges

The success of gene therapy for Leber Congenital Amaurosis (LCA2) due to defects in the *RPE65* gene and subsequent FDA and EMA approval of Luxturna (voretigene neparvovec) have sparked hope that many other autosomal recessive retinal (and neurological) diseases may also be treated using a vector-based gene delivery approach.[21,22] USH syndrome was no exception and several laboratories embarked on USH gene transfer studies in animal models. Conventional recombinant adeno-associated viral vectors (AAVs) contain a single-stranded DNA expression cassette, which includes a cell-specific or ubiquitous promoter, the transgene and polyadenylation signal, flanked by palindromic inverted terminal repeats at both ends. In contrast to other gene therapy systems, AAV vectors have low immunogenicity (especially when given subretinally), display long-lasting expression in postmitotic cells due to their ability to primarily persist as episomes, and efficiently transduce both photoreceptors and the retinal pigmented epithelium (RPE), where

most RP-associated genes are expressed.[23] Overall, AAV vectors hold tremendous potential to prevent retinal degeneration in USH syndrome. However, there are numerous challenges in the field, which become apparent by acknowledging the critical disease-specific parameters that account for the success of AAV-based gene augmentation therapy in RPE65 LCA. These include the availability of small and large animal models of RPE65-deficiency displaying a robust phenotype, a slow degenerative component of the disease accompanying a marked retinal dysfunction, a large window for treatment, and the lack of detrimental effects following long-term AAV-mediated transgene overexpression in RPE.[24] In contrast to ocular diseases in USH syndrome, the molecular basis of retinal dysfunction in LCA2 is well understood. Moreover, the lack of RPE65 causes a substantial loss of visual function in murine and canine animal models, as demonstrated by the significantly reduced or nonrecordable electroretinograms.[25–27] The marked loss of vision in animal models, which contrasts sharply with the remarkable preservation of retinal architecture, has allowed testing of long-term gene therapy effects.[28–30] The RPE65 protein is a 65 kDa retinoid isomerohydrolase, which plays an essential role in the retinoid visual cycle by converting all-trans-retinyl ester to 11-cis-retinol in RPE cells.[31–33] The 11-cis retinal chromophore deficiency in LCA2 photoreceptor outer segments can be corrected by restoring RPE65 enzyme to RPE, either by using a cell-specific human RPE65 promoter or a modified version of the ubiquitous chicken-beta actin promoter. Furthermore, one RPE cells interdigitates with 20 to 40 photoreceptor cells at the apical interface, amplifying the effects of gene therapy while minimizing the potential downside of nonuniform AAV-mediated transgene expression in neighboring RPE cells. Subretinal injection of an AAV2 vector delivering the canine wild-type RPE65 cDNA in dog models has produced long-term beneficial effects on visual function and protection from degeneration especially when treatment was initiated early.[29,30,34,35] Ultimately, the successful restoration of vision in a large animal model paved the way towards an AAV-based gene therapy for LCA2 patients.[36–40]

Unlike the case of RPE65-associated disease, the majority of the genetically engineered mouse models of USH syndrome do not exhibit a visual phenotype, although they all display hearing loss.[41,42] Some of these models were reported to display only a progressive retinal dysfunction with aging.[42] It has been suggested that the lack of a retinal phenotype in mouse models could result from a distinct species-specific cell pattern of gene expression or to morphologic differences between human and mouse photoreceptors, for example, mice lack the well-developed, actin-filled calyceal processes, the subcellular structures extending from the inner segments in frogs, pigs, zebrafish, and primates.[43,44] The absence of a robust ocular phenotype in animal models has severely limited the ability to perform therapeutic, preclinical proof-of-concept testing for vision loss

in USH syndrome. However, gene replacement, gene editing, and small molecule therapy have been used to preserve hearing in many USH animal models with positive results.[9,45–53] There are several recent, in-depth reviews of these therapies.[9,54,55] These data, combined with the progressive nature of the vision loss due to USH syndrome, have motivated a few companies to empirically study treatments on human subjects. One example is the lentiviral-mediated MYO7A gene delivery in USH1B.[56,57] This approach was shown to correct the melanosome mislocalization and opsin accumulation at the photoreceptor connecting cilium in the Myo7a-deficient shaker1 mouse model of USH1B.[57] Conventional AAV has a small packaging capacity compared with lentiviral vector (4.7 vs. 8.5 kb). MYO7A cDNA, which is expressed in photoreceptors and the RPE cells, is too large (~6.7 kb) to be packaged in AAV vectors.[58] Therefore, an equine infectious anemia virus-based lentiviral vector (equine infectious anemia virus-CMV-MYO7A, UshStat) was subretinally delivered to USH1B patients with the goal to prevent or slow down the RP progression (ClinicalTrials.gov identifier: NCT01505062). Long-term safety evaluation of this treatment in patients who received UshStat is currently being performed in a clinical trial (NCT02065011, results pending). Another clinical trial (UshTher) is currently underway, which uses a dual AAV vector approach to express MYO7A in the retina of patients following subretinal delivery (https://cordis.europa.eu/project/id/754848/it). Dual AAV vectors take advantage of the ability of the 2 distinct AAV genomes containing the 5′ and 3′ half of MYO7A transgene cDNA to undergo intermolecular concatamerization, followed by homologous recombination to reconstitute the whole large gene expression cassette.[59,60] To date, there has been no clinically positive data reported from either one of these trials.

The disease associated with much larger genes, for example, USH2A (cDNA~15.6 kb), may benefit from genome editing approaches, such as CRISPR-Cas9-sgRNAs, and those that promote exon skipping to restore functional protein restoration.[61–66] A splicing-modulating antisense oligonucleotide approach designed to cause in-frame skipping of the mutated exon 13 of the USH2A disease-associated transcripts results in a shortened but functional usherin protein, which localizes correctly in photoreceptors and hair cells.[66–68] Interestingly, recent studies have shown that zebrafish models of USH2A disease display early-onset retinal dysfunction, indicating they may represent useful tools to investigate the pathophysiology underlying USH syndrome and test therapeutic strategies.[69,70] Morpholino antisense oligonucleotides were shown to mediate *ush2a* exon 13 skipping and rescue the visual dysfunction in a zebrafish USH2A model.[66] This mutation-specific therapeutic strategy is being evaluated in a clinical trial using an antisense oligonucleotide (QR-421a) administered through intravitreal injections, with early evidence of efficacy reported

at the 3-month interim analysis (phase 1/2 Stellar trial of QR-421a, ProQR Therapeutics, Clinicaltrials.gov ID: NCT03780257).

Critical for the success of future therapeutic approaches is the generation and characterization of novel animal models that mimic the human ocular USH disease. These models will not only serve as preclinical platforms to test potential therapies, including viral-based gene delivery strategies, but can also be used to gain insight into the molecular mechanisms underlying retinal dysfunction and degeneration in USH syndrome. Interestingly, a pig model of USH1C lacking harmonin, USH1C (R31X), was recently reported to mimic the human disease, displaying combined deafness, changes in photoreceptor architecture, and significantly reduced visual function.[71]

USH syndrome has an estimated prevalence ranging from 4 to 17 cases per 100,000 individuals worldwide.[72,73] Approximately 98% of USH syndrome patients belong to either type 1 or type 2 subgroups of disease. In contrast, USH3 is relatively rare, accounting for ~2% of all cases.[74] However, it is the most prevalent form of USH disease in the Finnish population, where it represents an estimated 40% of all USH cases, and it also segregates to a high degree in patients of Ashkenazi-Jewish descent.[16,17,74] Through funding from the Usher III initiative, a team of researchers was brought together in 2007 to uncover the roles of CLRN1 in sensory organs and the pathology that ensues when the gene is defective. Several therapeutic approaches were developed as a result of this sustained effort, with the ultimate goal of treating USH3A patients. An offshoot of this effort also resulted in several NIH-funded grants specifically directed towards understanding the complex pathophysiology of USH3 in the retina and cochlea. The rest of this review will highlight important findings of this research, and discuss strategies for developing a potentially effective gene therapy approach for USH syndrome.

■ The USH Protein Network

The lack of therapeutic approaches to prevent vision loss in USH patients has motivated a considerable amount of research on the pathophysiology of disease. Fluorescence colocalization studies on tissue sections from different species and reciprocal pull-down assays suggest that all proteins encoded by USH genes interact with each other and organize into large complexes within specific subcellular regions in photoreceptors and hair cells.[75] They form highly dynamic networks, which consist of proteins from different classes with critical roles in transport, adhesion, and signaling processes in both sensory cell types. USH proteins include the actin-based motor protein myosin VIIa (MYO7A, USH1B); the cell-adhesion transmembrane proteins cadherin 23 (CDH23, USH1D), protocadherin 15 (PCDH15, USH1F), and

usherin (USH2A); the very-large G protein-coupled receptor-1 VLGR1 (ADGRV1, GPR98, USH2C); the calcium and integrin binding protein 2 (CIB2, USH1J); the PDZ-domain scaffolding proteins harmonin (USH1C) and whirlin (WHRN, USH2D); the ankyrin and SAM-domain containing scaffolding protein SANS (USH1G); the tetraspanin-like protein clarin-1 (CLRN1, USH3A). In the retina, USH proteins were found to concentrate in specialized regions of photoreceptor cells, the connecting cilium, calyceal processes, and periciliary membrane.[2] Some of the USH proteins were also detected at ribbon synapses in both photoreceptors and hair cells, which enable a rapid and sustained release of neurotransmitters necessary for the high frequency transmission of signals in sensory cells.[76]

The auditory system has often served as a mirror to gain insight into potential functions of USH proteins in the retina. In the cochlea, USH proteins were detected in the hair cell bundle, a mechanosensory antenna containing numerous F-actin rich stereocilia projecting from the apical surface of these auditory cells. As an elegant example, PCDH15 and CDH23 form both transient and permanent extracellular filamentous links between neighboring stereocilia, while harmonin, Sans, and myosin VIIa anchor these links to the intracellular actin core.[77–80] In adult stages, PCDH15 and CDH23 form tip links, suggested to act similar to gating springs and open the sensory mechanoelectrical transduction ion channels in response to mechanical stimuli provided by sound waves.[81,82] In the retina, USH1 proteins were detected at the fragile actin-rich calyceal processes in macaque, zebrafish, and humans, and may play a role in their development and maintenance.[43,83] Similar to the interstereociliary links found in the hair bundle, USH1 proteins may form and stabilize membrane-membrane connection sites linking the calyceal processes to photoreceptor outer segments, allowing them to withstand daily mechanical stress.[43] Consistent with this possibility, USH proteins organize and stabilize interactions between microvilli of absorptive intestinal cells.[84,85]

In contrast to USH1, the USH2 proteins usherin, VLGR1, and whirlin are mainly located in the periciliary ridge membrane complex region surrounding the photoreceptor connecting cilium, where they provide structural support and may also participate in vesicle docking with the plasma membrane during protein translocation to the outer segment.[63,86] Pathogenic mutations leading to dysfunction or absence of a particular USH protein were found to alter the localization of other members in the network, a property which has been exploited to evaluate the efficacy of gene therapy experiments in animal models.[87] Considering their subcellular localization to specific regions critical for transport and structural maintenance, careful AAV vector dosing and choice of regulatable promoters in gene therapy studies will be needed to ensure that long-term vector-mediated USH proteins expression mimics endogenous levels

and does not lead to detrimental effects on photoreceptors structure and function.[88] The use of novel penetrating AAV capsids with higher transduction efficiency may enable photoreceptor targeting following a surgically safer intravitreal approach, reducing the concentration of vector reaching the outer retina, and avoiding potential overexpression effects.[89,90]

■ CLRN1: an "Invisible" Tetraspanin Protein of Unknown Biological Function

A major challenge in the USH field lies in the difficulty of reliably detecting the USH proteins in the retina, due to limited epitope availability, a consequence of USH protein complexes and their low levels of expression. This aspect, combined with the lack of thoroughly validated antibody reagents and proper negative controls in certain cases, has generated conflicting information regarding the cellular expression pattern of some of the USH proteins in the retina. This is especially true for CLRN1, a tetraspanin-like glycoprotein whose biological function is currently unknown.[91] The main CLRN1 isoform encodes a 232 amino acid protein that contains a single glycosylation site at asparagine 48 (N48) in the first extracellular loop and a potential PDZ binding motif within its C-terminal end.[91,92] In vitro experiments using a hemagglutinin (HA)-epitope tagged CLRN1 have shown that following its overexpression in HEK cells, the protein concentrates at specific regions within plasma membrane and forms CLRN1-enriched microdomains, recruiting diverse proteins involved in cell-adhesion, focal adhesion, and tight junctions, with an essential role in organizing the actin cytoskeleton.[93]

In the cochlea, CLRN1 has been suggested to interact with USH1 proteins harmonin and PCDH15.[47,94] *Clrn1* knockout and the N48K knock-in mouse models of USH3 do not develop a retinal degeneration phenotype, but display an early-onset hearing loss and disorganized F-actin-rich stereocilia, indicating that one function of CLRN1 in the cochlea is to maintain the structural integrity of the hair bundle.[95–97] A zebrafish model further established that CLRN1 is as an essential hair bundle protein.[98] Other studies reported that CLRN1 absence also causes synaptic defects in cochlea hair cells.[47,94] Three recent studies have shown that gene therapy can successfully prevent hearing loss in different mouse models of USH3 following early postnatal AAV-mediated *Clrn1* cochlear delivery, when the sensory hair cell structure is preserved.[47–49] These proof of principle studies raise hope that AAV-mediated CLRN1 expression to the correct retinal target cells will also be successful, providing that therapy is initiated before significant structural damage to photoreceptor cells occurs.

Studies aimed to define the cellular expression of endogenous CLRN1 in the eye detected it either in the inner or outer retinal cells, or both, depending on the method used (in situ hybridization for mRNA or immunostaining for protein).[97,99–101] Localization was further confounded by differences in RNA hybridization probes or antibodies used, variability in sample processing conditions, as well as the particular species analyzed. In one study, *Clrn1* transcripts were transiently detected during postnatal development in the inner retina by in situ hybridization (ISH), in a pattern consistent with progenitor cells and Müller glia expression.[97] By using rodless aged rd1 mice and laser capture microdissection, that study further showed that in adult mouse retinas *Clrn1* mRNA was confined to the inner nuclear layer, detectable only by RT-PCR. In the same year, another group reported that CLRN1 protein was expressed in photoreceptor synapses and connecting cilium regions following immunostaining of the mouse retina with a custom made anti-CLRN1 antibody.[101] In zebrafish, CLRN1 was detected in both the inner and outer retina.[99] However, several studies concluded that endogenous CLRN1 protein remains below the levels of detection of immunostaining.[47,49,97,100] Recently, we have generated and characterized a novel N-terminus epitope-tagged CLRN1 knock-in mouse to facilitate the detection of endogenous CLRN1 protein expression in retinal sections with a high affinity anti-HA antibody.[100] We noted a similar background labeling pattern in both HA-tagged CLRN1 knock-in and the C57BL/6J controls. Importantly, the HA-tagged CLRN1 in this mouse was only detectable by western blot analysis of retinal homogenates, and was expressed continuously during postnatal development and adulthood.[100] The HA-tagged CLRN1 protein in retinal sections could only be successfully detected following its viral-mediated overexpression using a subretinal or intravitreal delivery approach.[100,102]

The availability of single-cell RNA sequencing (scRNAseq) technology has provided an unprecedented opportunity to firmly map the localization of *CLRN1* and other USH gene transcripts in the retina in multiple species, providing a complementary tool to immunodetection.[103,104] In our recently published study, by using scRNAseq analysis of large data sets, we bypassed the challenges of immunostaining experiments, and convincingly demonstrated that *CLRN1* transcripts in adult retina are present in Müller glia in human, mouse and non-human primates.[100] Müller glia enrichment of *CLRN1* transcripts in the retina has been corroborated in human samples by other scRNAseq studies.[105–108] Interestingly, scRNAseq data sets have provided evidence that *HARMONIN* (USH1C), *CDH23* (USH1D), and *SANS* (USH1G) transcripts are also present Müller glia, supporting the need to consider the involvement of multiple cell types in USH disease.[108] Furthermore, by using the highly sensitive RNAscope ISH assay we detected *CLRN1* transcripts in the inner retina and not photoreceptors, both in the adult mouse and human tissue, a result consistent with the

scRNAseq data.[100] No signal was detected when the ISH *Clrn1* target probes were used on *Clrn1* knockout mouse sections, confirming the high specificity of the assay.[100] We have recently examined *CLRN1* mRNA expression in the pig retina using a similar RNAscope ISH chromogenic assay. We determined that *CLRN1* transcripts are abundantly expressed early in postnatal development, before the photoreceptor outer segments are fully developed, concentrating within the middle part of the inner nuclear layer of piglet retinas (Fig. 1). Taken together, these experiments provide solid evidence that *CLRN1* transcripts are expressed in the inner retina in multiple species, and highlight the complexities inherent in understanding the molecular pathophysiology of vision loss in USH syndrome and potentially, a novel biology associated with retinal degeneration caused by Müller glia dysfunction. However, these studies do not completely rule out the possibility that CLRN1 might also be expressed in photoreceptor cells at very low levels, undetectable with our current technologies.

The potential roles of CLRN1 in protein scaffolding and signaling within the retina, as well as its specific molecular impact on Müller glia and photoreceptor neurons are currently unknown. Müller glia are involved in many different aspects of retinal biology, including neuronal development, modulation of neuronal synaptic activity and structural support of photoreceptor cells, and there are species-specific differences between the density and architecture of these cells in the retina.[109,110] Previous studies have also suggested a possible function for Müller glia as radial tension sensors, showing that these extraordinary cells are sensitive to mechanically enforced changes in retinal structure.[111,112] They have been compared with taut springs, providing mechanical resilience to the retina, and protecting the retina from ripping apart.[111] Under normal

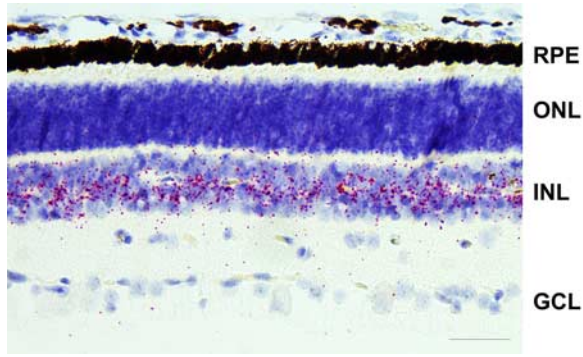

Figure 1. *Detection of CLRN1 transcripts in piglet retina at postnatal day P2 using the RNAscope ISH assay (red punctate staining). GCL indicates ganglion cell layer; INL, inner nuclear layer; ISH, in situ hybridization; ONL, outer nuclear layer; RPE, retinal pigment epithelium. The paraffin-embedded retina tissue sections were counterstained with Gill hematoxylin to visualize nuclei (blue). Scale bar: 20 μm.*

physiological conditions, the retina is subjected to daily mechanical stress, including during its postnatal expansion. As a tetraspanin, CLRN1 may be involved in maintaining adhesion links between Müller glia and retinal neurons, protecting photoreceptors from mechanical stress, thus enabling their proper function throughout development and with aging.

USH3 patients often display a severe impairment in retinal function at very early stages of the disease, with electroretinogram amplitudes being notably decreased in children as young as 3 to 5 years of age.[16] Novel animal models of USH3 that mimic human retinal disease could be used to answer several critical questions with regard to gene therapy approaches. First, is presymptomatic transgene expression of CLRN1 required within photoreceptors, Müller glia, or both cell types to prevent blindness. Second, does ectopic CLRN1 expression in either photoreceptors or Müller glia actually promote degeneration. This could happen if exogenous CLRN1 protein prevents normal USH complexes formation in those cells. Finally, does CLRN1 delivery in adult stages, following symptomatic onset, restore vision loss and slow down the disease progression in USH3 patients. A variety of AAV-based gene therapy vector capsids and promoters targeting either photoreceptors or Müller glia are already available, and more are currently being generated and continuously optimized.[23,89,113–116] The development and characterization of large animal models of USH syndrome is urgently needed in the field, as it will contribute greatly to the advancement of our understanding of the pathophysiology of this disease, and critically, provide an opportunity to study the cellular autonomy of retinal degeneration in USH patients.

■ Conclusions

On the surface, gene replacement appears to be a logical path to treat autosomal recessive retinal pathologies. This has led to the many laboratories and companies moving directly to gene therapeutic strategies to treat certain USH subtypes. The success of this path is yet to be determined. This review has highlighted several hurdles that should be overcome to optimize development of gene therapy towards specific inherited diseases. These fall into 2 general areas, (1) understanding the molecular pathology of the specific disease, and (2) developing gene therapy vectors that safely reach the target cells and express in a manner that effectively corrects biochemical defects without causing collateral damage. In LCA2, the detailed understanding of RPE65 role in vision, the simplicity of the gene structure and availability of both engineered and naturally occurring small (mouse) and large (dog) animal models enabled many researchers to successfully treat these animals, leading to the regulatory approvals of Luxturna. This review and others painfully

demonstrate the complexities inherent in understanding the molecular pathophysiology of vision loss secondary to USH disease. Both the lack of solid understanding of the normal function of the associated proteins, and how the omission of these proteins specifically impacts the retina, limit the ability to develop USH therapies. This is true for all forms of USH, but perhaps is most evident for USH3, caused by mutations in an "invisible" CLRN1 protein. A key tool necessary to reveal this information is the generation of suitable animal models displaying a robust visual dysfunction and retinal degeneration phenotype. These models will allow basic scientists to better understand the molecular basis of disease and translational laboratories to develop effective therapies.

■ Acknowledgments

The authors thank the Lauhakangas family, whose philanthropic support helped make this research possible. They would also like to acknowledge the support of Usher III Initiative Inc.

Supported by funding from National Institutes of Health grant EY026559, BrightFocus Foundation M2017035, Foundation Fighting Blindness awards TA-NMT-0621-0811-UFL and PPA-0617-0718, and an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology of University of Florida.
The authors declare that they have no conflicts of interest to disclose.

■ References

1. Toms M, Pagarkar W, Moosajee M. Usher syndrome: clinical features, molecular genetics and advancing therapeutics. *Ther Adv Ophthalmol*. 2020;12:2515841420952194.
2. Mathur P, Yang J. Usher syndrome: hearing loss, retinal degeneration and associated abnormalities. *Biochim Biophys Acta*. 2015;1852:406–420.
3. Petit C. Usher syndrome: from genetics to pathogenesis. *Annu Rev Genomics Hum Genet*. 2001;2:271–297.
4. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006;368:1795–1809.
5. Usher CH. On the inheritance of retinitis pigmentosa; with notes of cases. *Roy Lond Ophthalmol Hosp Rep*. 1914;19:130–236.
6. Von Graefe A. Exceptionelles verrhalten des gesichtsfeldes bei pigmentenartung der netzhaut. *Von Graefes Arch Ophtlalmol*. 1858;4:250–253.
7. Liebreich R. Abkunft aus Ehen unter Blutsverwandten als Grund von Retinitis pigmentosa. *Deutsch Klin*. 1861;13:53–55.
8. Kimberling WJ, Moller C. Clinical and molecular genetics of Usher syndrome. *J Am Acad Audiol*. 1995;6:63–72.
9. Geleoc GGS, El-Amraoui A. Disease mechanisms and gene therapy for Usher syndrome. *Hear Res*. 2020;394:107932.
10. Jacobson SG, Cideciyan AV, Gibbs D, et al. Retinal disease course in Usher syndrome 1B due to MYO7A mutations. *Invest Ophthalmol Vis Sci*. 2011;52:7924–7936.

11. Subira O, Catala-Mora J, Diaz-Cascajosa J, et al. Retinal findings in pediatric patients with Usher syndrome Type 1 due to mutations in MYO7A gene. *Eye (Lond)*. 2020; 34:499–506.
12. Fishman GA, Bozbeyoglu S, Massof RW, et al. Natural course of visual field loss in patients with Type 2 Usher syndrome. *Retina*. 2007;27:601–608.
13. Herrera W, Aleman TS, Cideciyan AV, et al. Retinal disease in Usher syndrome III caused by mutations in the clarin-1 gene. *Invest Ophthalmol Vis Sci*. 2008;49:2651–2660.
14. Ratnam K, Vastinsalo H, Roorda A, et al. Cone structure in patients with usher syndrome type III and mutations in the Clarin 1 gene. *JAMA Ophthalmol*. 2013;131: 67–74.
15. Aller E, Jaijo T, Oltra S, et al. Mutation screening of USH3 gene (clarin-1) in Spanish patients with Usher syndrome: low prevalence and phenotypic variability. *Clin Genet*. 2004;66:525–529.
16. Pakarinen L, Tuppurainen K, Laippala P, et al. The ophthalmological course of Usher syndrome type III. *Int Ophthalmol*. 1995;19:307–311.
17. Ness SL, Ben-Yosef T, Bar-Lev A, et al. Genetic homogeneity and phenotypic variability among Ashkenazi Jews with Usher syndrome type III. *J Med Genet*. 2003; 40:767–772.
18. Abbott JA, Guth E, Kim C, et al. The Usher syndrome type IIIB histidyl-tRNA synthetase mutation confers temperature sensitivity. *Biochemistry*. 2017;56:3619–3631.
19. Puffenberger EG, Jinks RN, Sougnez C, et al. Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One*. 2012;7: e28936.
20. Nolen RM, Hufnagel RB, Friedman TB, et al. Atypical and ultra-rare Usher syndrome: a review. *Ophthalmic Genet*. 2020;41:401–412.
21. Maguire AM, Bennett J, Aleman EM, et al. Clinical perspective: treating RPE65-associated retinal dystrophy. *Mol Ther*. 2021;29:442–463.
22. Gao J, Hussain RM, Weng CY. Voretigene neparvovec in retinal diseases: a review of the current clinical evidence. *Clin Ophthalmol*. 2020;14:3855–3869.
23. Buck TM, Wijnholds J. Recombinant adeno-associated viral vectors (rAAV)-vector elements in ocular gene therapy clinical trials and transgene expression and bioactivity assays. *Int J Mol Sci*. 2020;21:4197.
24. Pierce EA, Bennett J. The status of RPE65 gene therapy trials: safety and efficacy. *Cold Spring Harb Perspect Med*. 2015;5:a017285.
25. Narfstrom K, Wrigstad A, Nilsson SE. The Briard dog: a new animal model of congenital stationary night blindness. *Br J Ophthalmol*. 1989;73:750–756.
26. Pang JJ, Chang B, Hawes NL, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). *Mol Vis*. 2005;11:152–162.
27. Veske A, Nilsson SE, Narfstrom K, et al. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. *Genomics*. 1999;57:57–61.
28. Pang JJ, Chang B, Kumar A, et al. Gene therapy restores vision-dependent behavior as well as retinal structure and function in a mouse model of RPE65 Leber congenital amaurosis. *Mol Ther*. 2006;13:565–572.
29. Narfstrom K, Katz ML, Ford M, et al. In vivo gene therapy in young and adult RPE65-/- dogs produces long-term visual improvement. *J Hered*. 2003;94:31–37.
30. Cideciyan AV, Jacobson SG, Beltran WA, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci USA*. 2013;110:E517–E525.
31. Redmond TM, Yu S, Lee E, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet*. 1998;20:344–351.
32. Jin M, Li S, Moghrabi WN, Sun H, et al. Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. *Cell*. 2005;122:449–459.

33. Moiseyev G, Chen Y, Takahashi Y, et al. RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc Natl Acad Sci USA*. 2005;102:12413–12418.
34. Jacobson SG, Acland GM, Aguirre GD, et al. Safety of recombinant adeno-associated virus type 2-RPE65 vector delivered by ocular subretinal injection. *Mol Ther*. 2006; 13:1074–1084.
35. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. 2001;28:92–95.
36. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci USA*. 2008;105:15112–15117.
37. Cideciyan AV, Hauswirth WW, Aleman TS, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. *Hum Gene Ther*. 2009;20:999–1004.
38. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther*. 2008;19:979–990.
39. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2240–2248.
40. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2231–2239.
41. Williams DS. Usher syndrome: animal models, retinal function of Usher proteins, and prospects for gene therapy. *Vision Res*. 2008;48:433–441.
42. Tian G, Lee R, Ropelewski P, et al. Impairment of vision in a mouse model of Usher syndrome type III. *Invest Ophthalmol Vis Sci*. 2016;57:866–875.
43. Sahly I, Dufour E, Schietroma C, et al. Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent from mice. *J Cell Biol*. 2012;199:381–399.
44. El-Amraoui A, Sahly I, Picaud S, et al. Human Usher 1B/mouse shaker-1: the retinal phenotype discrepancy explained by the presence/absence of myosin VIIA in the photoreceptor cells. *Hum Mol Genet*. 1996;5:1171–1178.
45. Alagramam KN, Gopal SR, Geng R, et al. A small molecule mitigates hearing loss in a mouse model of Usher syndrome III. *Nat Chem Biol*. 2016;12:444–451.
46. Lentz JJ, Pan B, Ponnath A, et al. Direct delivery of antisense oligonucleotides to the middle and inner ear improves hearing and balance in Usher mice. *Mol Ther*. 2020; 28:2662–2676.
47. Dulon D, Papal S, Patni P, et al. Clarin-1 gene transfer rescues auditory synaptopathy in model of Usher syndrome. *J Clin Invest*. 2018;128:3382–3401.
48. Gyorgy B, Meijer EJ, Ivanchenko MV, et al. Gene transfer with AAV9-PHP.B rescues hearing in a mouse model of Usher syndrome 3A and transduces hair cells in a non-human primate. *Mol Ther Methods Clin Dev*. 2019;13:1–13.
49. Geng R, Omar A, Gopal SR, et al. Modeling and preventing progressive hearing loss in Usher syndrome III. *Sci Rep*. 2017;7:13480.
50. Isgrig K, Shteamer JW, Belyantseva IA, et al. Gene therapy restores balance and auditory functions in a mouse model of Usher syndrome. *Mol Ther*. 2017;25:780–791.
51. Emptoz A, Michel V, Lelli A, et al. Local gene therapy durably restores vestibular function in a mouse model of Usher syndrome type 1G. *Proc Natl Acad Sci USA*. 2017;114:9695–9700.
52. Lentz JJ, Jodelka FM, Hinrich AJ, et al. Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness. *Nat Med*. 2013;19:345–350.
53. Gopal SR, Lee YT, Stepanyan R, et al. Unconventional secretory pathway activation restores hair cell mechanotransduction in an USH3A model. *Proc Natl Acad Sci USA*. 2019;116:11000–11009.
54. Delmaghani S, El-Amraoui A. Inner ear gene therapies take off: current promises and future challenges. *J Clin Med*. 2020;9:2309.

55. Akil O. Dual and triple AAV delivery of large therapeutic gene sequences into the inner ear. *Hear Res*. 2020;394:107912.
56. Zallocchi M, Binley K, Lad Y, et al. EIAV-based retinal gene therapy in the shaker1 mouse model for Usher syndrome type 1B: development of UshStat. *PLoS One*. 2014;9:e94272.
57. Hashimoto T, Gibbs D, Lillo C, et al. Lentiviral gene replacement therapy of retinas in a mouse model for Usher syndrome type 1B. *Gene Ther*. 2007;14:584–594.
58. Lopes VS, Williams DS. Gene therapy for the retinal degeneration of Usher syndrome caused by mutations in MYO7A. *Cold Spring Harb Perspect Med*. 2015;5: a017319.
59. Dyka FM, Boye SL, Chiodo VA, et al. Dual adeno-associated virus vectors result in efficient in vitro and in vivo expression of an oversized gene, MYO7A. *Hum Gene Ther Methods*. 2014;25:166–177.
60. Trapani I, Colella P, Sommella A, et al. Effective delivery of large genes to the retina by dual AAV vectors. *EMBO Mol Med*. 2014;6:194–211.
61. Pendse N, Lamas V, Pawlyk B, et al. CRISPR/Cas based evaluation of the therapeutic potential for USH2A associated diseases. *Mol Ther*. 2019;27:317–318.
62. Tachida Y, Levy JM, Pendse N, et al. Efficient identification of therapeutic base editing candidates to correct human mutations in the USH2A gene. *Mol Ther*. 2020; 28:324–324.
63. Toualbi L, Toms M, Moosajee M. USH2A-retinopathy: from genetics to therapeutics. *Exp Eye Res*. 2020;201:108330.
64. Sanjurjo-Soriano C, Erkilic N, Baux D, et al. Genome editing in patient iPSCs corrects the most prevalent USH2A mutations and reveals intriguing mutant mRNA expression profiles. *Mol Ther Methods Clin Dev*. 2020;17:156–173.
65. Xue K, MacLaren RE. Antisense oligonucleotide therapeutics in clinical trials for the treatment of inherited retinal diseases. *Expert Opin Investig Drugs*. 2020;29:1163–1170.
66. Dulla K, Slijkerman R, van Diepen HC, et al. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations. *Mol Ther*. 2021;29:2441–2455.
67. Pendse ND, Lamas V, Pawlyk BS, et al. In vivo assessment of potential therapeutic approaches for USH2A-associated diseases. *Adv Exp Med Biol*. 2019;1185:91–96.
68. Pendse N, Lamas V, Maeder M, et al. Exon 13-skipped USH2A protein retains functional integrity in mice, suggesting an exo-skipping therapeutic approach to treat USH2A-associated disease. *BioRxiv*. 2020. doi: https://doi.org/10.1101/2020.02.04.934240
69. Dona M, Slijkerman R, Lerner K, et al. Usherin defects lead to early-onset retinal dysfunction in zebrafish. *Exp Eye Res*. 2018;173:148–159.
70. Han S, Liu X, Xie S, et al. Knockout of ush2a gene in zebrafish causes hearing impairment and late onset rod-cone dystrophy. *Hum Genet*. 2018;137:779–794.
71. Grotz S, Schäfer J, Wunderlich KA, et al. Early disruption of photoreceptor cell architecture and loss of vision in a humanized pig model of Usher syndrome. *BioRxiv*. 2021. doi: https://doi.org/10.1101/2021.05.31.446123
72. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. *J Chronic Dis*. 1983;36:595–603.
73. Kimberling WJ, Hildebrand MS, Shearer AE, et al. Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genet Med*. 2010;12:512–516.
74. Pakarinen L, Karjalainen S, Simola KO, et al. Usher's syndrome type 3 in Finland. *Laryngoscope*. 1995;105:613–617.
75. Sorusch N, Wunderlich K, Bauss K, et al. Usher syndrome protein network functions in the retina and their relation to other retinal ciliopathies. *Adv Exp Med Biol*. 2014;801:527–533.

76. Reiners J, van Wijk E, Marker T, et al. Scaffold protein harmonin (USH1C) provides molecular links between Usher syndrome type 1 and type 2. *Hum Mol Genet*. 2005;14: 3933–3943.
77. El-Amraoui A, Petit C. Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. *J Cell Sci*. 2005;118 (pt 20):4593–4603.
78. Yu IM, Planelles-Herrero VJ, Sourigues Y, et al. Myosin 7 and its adaptors link cadherins to actin. *Nat Commun*. 2017;8:15864.
79. Boeda B, El-Amraoui A, Bahloul A, et al. Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *EMBO J*. 2002;21:6689–6699.
80. Siemens J, Kazmierczak P, Reynolds A, et al. The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions. *Proc Natl Acad Sci USA*. 2002;99:14946–14951.
81. Bonnet C, El-Amraoui A. Usher syndrome (sensorineural deafness and retinitis pigmentosa): pathogenesis, molecular diagnosis and therapeutic approaches. *Curr Opin Neurol*. 2012;25:42–49.
82. Siemens J, Lillo C, Dumont RA, et al. Cadherin 23 is a component of the tip link in hair-cell stereocilia. *Nature*. 2004;428:950–955.
83. Schietroma C, Parain K, Estivalet A, et al. Usher syndrome type 1-associated cadherins shape the photoreceptor outer segment. *J Cell Biol*. 2017;216:1849–1864.
84. Crawley SW, Shifrin DA Jr, Grega-Larson NE, et al. Intestinal brush border assembly driven by protocadherin-based intermicrovillar adhesion. *Cell*. 2014;157:433–446.
85. Crawley SW, Weck ML, Grega-Larson NE, et al. ANKS4B is essential for intermicrovillar adhesion complex formation. *Dev Cell*. 2016;36:190–200.
86. Yang J, Wang L, Song H, et al. Current understanding of Usher syndrome type II. *Front Biosci*. 2012;17:1165–1183.
87. Zou J, Luo L, Shen Z, et al. Whirlin replacement restores the formation of the USH2 protein complex in whirlin knockout photoreceptors. *Invest Ophthalmol Vis Sci*. 2011;52:2343–2351.
88. Santiago CP, Keuthan CJ, Boye SL, et al. A drug-tunable gene therapy for broad-spectrum protection against retinal degeneration. *Mol Ther*. 2018;26:2407–2417.
89. Byrne LC, Day TP, Visel M, et al. In vivo-directed evolution of adeno-associated virus in the primate retina. *JCI Insight*. 2020;5:e135112.
90. Petrs-Silva H, Dinculescu A, Li Q, et al. Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. *Mol Ther*. 2011;19:293–301.
91. Adato A, Vreugde S, Joensuu T, et al. USH3A transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses. *Eur J Hum Genet*. 2002;10:339–350.
92. Vastinsalo H, Jalkanen R, Dinculescu A, et al. Alternative splice variants of the USH3A gene Clarin 1 (CLRN1). *Eur J Hum Genet*. 2011;19:30–35.
93. Tian G, Zhou Y, Hajkova D, et al. Clarin-1, encoded by the Usher Syndrome III causative gene, forms a membranous microdomain: possible role of clarin-1 in organizing the actin cytoskeleton. *J Biol Chem*. 2009;284:18980–18993.
94. Ogun O, Zallocchi M. Clarin-1 acts as a modulator of mechanotransduction activity and presynaptic ribbon assembly. *J Cell Biol*. 2014;207:375–391.
95. Geng R, Geller SF, Hayashi T, et al. Usher syndrome IIIA gene clarin-1 is essential for hair cell function and associated neural activation. *Hum Mol Genet*. 2009;18:2748–2760.
96. Geng R, Melki S, Chen DH, et al. The mechanosensory structure of the hair cell requires clarin-1, a protein encoded by Usher syndrome III causative gene. *J Neurosci*. 2012;32:9485–9498.
97. Geller SF, Guerin KI, Visel M, et al. CLRN1 is nonessential in the mouse retina but is required for cochlear hair cell development. *PLoS Genet*. 2009;5:e1000607.

98. Gopal SR, Chen DH, Chou SW, et al. Zebrafish models for the mechanosensory hair cell dysfunction in Usher syndrome 3 reveal that clarin-1 is an essential hair bundle protein. *J Neurosci*. 2015;35:10188–10201.
99. Phillips JB, Vastinsalo H, Wegner J, et al. The cone-dominant retina and the inner ear of zebrafish express the ortholog of CLRN1, the causative gene of human Usher syndrome type 3A. *Gene Expr Patterns*. 2013;13:473–481.
100. Xu L, Bolch SN, Santiago CP, et al. Clarin-1 expression in adult mouse and human retina highlights a role of Muller glia in Usher syndrome. *J Pathol*. 2020;250:195–204.
101. Zallocchi M, Meehan DT, Delimont D, et al. Localization and expression of clarin-1, the Clrn1 gene product, in auditory hair cells and photoreceptors. *Hear Res*. 2009;255:109–120.
102. Dinculescu A, Stupay RM, Deng WT, et al. AAV-mediated clarin-1 expression in the mouse retina: implications for USH3A gene therapy. *PLoS One*. 2016;11:e0148874.
103. Peng YR, Shekhar K, Yan W, et al. Molecular classification and comparative taxonomics of foveal and peripheral cells in primate retina. *Cell*. 2019;176:1222–1237.
104. Voigt AP, Mullin NK, Stone EM, et al. Single-cell RNA sequencing in vision research: Insights into human retinal health and disease. *Prog Retin Eye Res*. 2020:100934.
105. Hu Y, Wang X, Hu B, et al. Dissecting the transcriptome landscape of the human fetal neural retina and retinal pigment epithelium by single-cell RNA-seq analysis. *PLoS Biol*. 2019;17:e3000365.
106. Lukowski SW, Lo CY, Sharov AA, et al. A single-cell transcriptome atlas of the adult human retina. *EMBO J*. 2019;38:e100811.
107. Orozco LD, Chen HH, Cox C, et al. Integration of eQTL and a single-cell atlas in the human eye identifies causal genes for age-related macular degeneration. *Cell Rep*. 2020;30:1246–1259.e1246.
108. Cowan CS, Renner M, De Gennaro M, et al. Cell types of the human retina and its organoids at single-cell resolution. *Cell*. 2020;182:1623–1640.
109. Bringmann A, Pannicke T, Grosche J, et al. Muller cells in the healthy and diseased retina. *Prog Retin Eye Res*. 2006;25:397–424.
110. Distler C, Dreher Z. Glia cells of the monkey retina—II. Muller cells. *Vision Res*. 1996;36:2381–2394.
111. MacDonald RB, Randlett O, Oswald J, et al. Muller glia provide essential tensile strength to the developing retina. *J Cell Biol*. 2015;210:1075–1083.
112. Lindqvist N, Liu Q, Zajadacz J, et al. Retinal glial (Muller) cells: sensing and responding to tissue stretch. *Invest Ophthalmol Vis Sci*. 2010;51:1683–1690.
113. Klimczak RR, Koerber JT, Dalkara D, et al. A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Muller cells. *PLoS One*. 2009;4:e7467.
114. Pellissier LP, Hoek RM, Vos RM, et al. Specific tools for targeting and expression in Muller glial cells. *Mol Ther Methods Clin Dev*. 2014;1:14009.
115. Juttner J, Szabo A, Gross-Scherf B, et al. Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates and humans. *Nat Neurosci*. 2019;22:1345–1356.
116. Korecki AJ, Cueva-Vargas JL, Fornes O, et al. Human MiniPromoters for ocular-rAAV expression in ON bipolar, cone, corneal, endothelial, Muller glial, and PAX6 cells. *Gene Ther*. 2021;28:351–372.