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Original Article

Dual AAV-based *PCDH15* gene therapy achieves sustained rescue of visual function in a mouse model of Usher syndrome 1F

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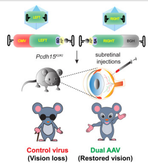
Mutations in the PCDH15 gene, encoding protocadherin-15, are among the leading causes of Usher syndrome type 1 (USH1F), and account for up to 12% USH1 cases worldwide. A founder truncating variant of *PCDH15* has a ~2% carrier frequency in Ashkenazi Jews accounting for nearly 60% of their USH1 cases. Although cochlear implants can restore hearing perception in USH1 patients, presently there are no effective treatments for the vision loss due to retinitis pigmentosa. We established a founder allele-specific *Pcdh15* knockin mouse model as a platform to ascertain therapeutic strategies. Using a dual-vector approach to circumvent the size limitation of adeno-associated virus, we observed robust expression of exogenous *PCDH15* in the retinae of *Pcdh15*$^{fl/fl}$ mice, sustained recovery of electroretinogram amplitudes and key retinoid oxime, substantially improved light-dependent translocation of phototransduction proteins, and enhanced levels of retinal pigment epithelium-derived enzymes. Thus, our data raise hope and pave the way for future gene therapy trials in USH1F subjects.

Graphical abstract



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Introduction

Usher syndrome (USH), a clinically and genetically heterogeneous disorder, results in hearing loss and pre-pubertal onset of retinitis pigmentosa (RP) with or without severe vestibular dysfunction.[1,2] Globally, USH accounts for 3%–6% of congenitally deaf individuals, ~50% of all hereditary deaf-blindness cases, and 8%–33% of patients with RP.[3,4,5] A genetic screening study in children with hearing loss in Oregon found that 11% had pathogenic variants in genes associated with USH and estimated that the prevalence may be as high as 1:6,000,[6] which would translate into 255,000 to 1.34 million USH cases worldwide. Hearing perception in USH subjects greatly improves with prosthetic cochlear implants usually done in early childhood.[4,7] However, currently there is no effective treatment for the vision loss due to RP, highlighting an unmet need for personalized treatment options, including gene therapy.[8,9]

Among the known genetic causes of USH, variants in *PCDH15*, encoding a large calcium-dependent cell-cell adhesion molecule termed protocadherin-15, are one of the leading causes of USH type 1 in the United States. The p.Arg245∗ founder variant of *PCDH15* (*USH1F*) has ~2% carrier frequency among Ashkenazi Jews, and accounts for nearly 60% of their USH1 cases.[4,10] USH1F subjects suffer with congenital hearing loss, vestibular areflexia, and progressive retinal degeneration, leading to severe vision loss with macular atrophy by their mid-fifties.[11] In the human retina, protocadherin-15 is expressed in the inner segment (IS) of photoreceptors, inner and outer plexiform layers, and retinal ganglion cells.[1,12] Higher-resolution imaging in non-human primates highlighted the preferential expression in the calyceal processes at the junction of IS and outer segment (OS) of photoreceptors.[13,14,15]

In the mouse retina, protocadherin-15 is localized to the outer limiting membrane and ISs of photoreceptors, retinal ganglion layer, Müller glia, and the retinal pigment epithelium (RPE).[11,16] Several *Pcdh15* homozygous mutant lines have shown a reduction of electroretinogram (ERG) a- and b-wave amplitudes (~40%) from very early age.[11,17] We recently reported that the homozygous *Pcdh15*^{GT200} (*Pcdh15*$^{fl/fl}$) henceforth; the mouse equivalent of the human p.Arg245∗ founder variant) mutant mice have visual deficits, aberrant light-dependent translocation of the phototransduction cascade proteins, arrestin and transducin, reduced expression of RPE-specific retinoid processing enzymes, RPE65 and CRALBP, and reduced retinal oxime with no apparent degeneration of their photoreceptor cells on the C57BL/6J background.[11] Hence, we utilized this patient variant equivalent mouse model for testing the efficacy of gene therapy to rescue the above-mentioned functional, anatomical, and biochemical deficits.

Adeno-associated viruses (AAVs) are the preferred vector of choice for retinal gene therapy due to a favorable safety profile, high transduction capacity, excellent tropism for photoreceptor and RPE cells, and long-term gene expression of the transgene.[2,18,19,20,21] The first *in vivo* gene therapy approved by the Food and Drug Administration in the US was for the retinal inherited Leber congenital amaurosis type 2 disorder which utilized an AAV2/2 vector to deliver a functional copy of the *RPE65* gene.[21] However, the DNA packaging capacity of AAVs (~4.7 kb) limits its applicability when developing gene replacement therapies for larger genes such as *PCDH15* (cDNA ~5.7 kb). Here, we overcame this size limitation by employing the dual-AAV system and adapting two different splicing recombination strategies to split the *PCDH15* gene into two halves and packaging them into separate AAV vectors.[23,24] We tested the efficacy of our strategies in heterologous cells and in the retinae of *Pcdh15*$^{fl/fl}$ mutant mice. We showed restoration of *PCDH15* expression, along with the sustained rescue of above-mentioned functional deficits in *Pcdh15*$^{fl/fl}$ mutant mice, thus expanding the scope of potential dual-AAV-based gene therapy to preserve vision in USH1F patients.

Section snippets

Development of dual-AAV-based *PCDH15* gene delivery strategy

To develop gene-replacement-based treatment for vision impairment in USH1F subjects, we used our recently reported *Pcdh15*$^{fl/fl}$ mice,[11] and designed a AAV-based gene augmentation strategy.[23] In humans, the coding sequence for *PCDH15* is ~5.7 kb, too large to fit in a single AAV vector, therefore, to circumvent this limitation, we designed and compared a dual-vector approach using hybrid and trans-splicing recombination strategies.[23] The longest human isoform of the *PCDH15* (ENST00000373957.7),...

Discussion

Here, we demonstrated that efficient gene delivery into the neurosensory retina with the dual-AAV vectors was able to rescue visual function in a patient-relevant mouse model of USH1F. We previously reported that mice homozygous for p.Arg250∗ truncating variant of *Pcdh15* have significantly attenuated ERG amplitudes, and deficits in their visual cycle and retinoid synthesis, without overt cell loss.[11] The *pcdh15b* zebrafish mutants also displayed vision deficits and structural deficits in the...

Animal model

All animal studies were conducted in accordance with the ARRIVE guidelines and ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and after approval by the University of Maryland Baltimore Institutional Animal Care and Use Committee. For gene augmentation studies, we used *Pcdh15* p.Arg250∗ knockin (equivalent to human Arg245∗; *Pcdh15*$^{fl/fl}$) mice.[11] Mice were housed in a facility with a 12 h...

Data and code availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request....

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Author contributions

Z.M.A. and L.S.C. designed and conceived the project. Sehar Riaz,...

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