Proposed therapy, developed in a Pcdh15-deficient mouse, for progressive loss of vision in human Usher Syndrome

Summary

A preclinical study using exogenous retinoids in a novel Usher syndrome 1F mouse model reveals a possible therapy to treat mutant PCDH15-mediated visual dysfunction.

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Abstract

Usher syndrome type I (USH1) is characterized by deafness, vestibular areflexia and progressive retinal degeneration. The protein-truncating p.Arg245* founder variant of PCDH15 (USH1F) has an ~2% carrier frequency amongst Ashkenazi Jews accounting for ~60% of their USH1 cases. Here, longitudinal phenotyping in thirteen USH1F individuals revealed progressive retinal degeneration, leading to severe vision loss with macular atrophy by the sixth decade. Half of the affected individuals were legally blind by their mid-fifties. The mouse Pcdh15R250X variant is equivalent to human p.Arg245*. Homozygous Pcdh15R250X mice also have visual deficits and aberrant light-dependent translocation of the phototransduction cascade proteins, arrestin and transducin. Retinal pigment epithelium- (RPE) specific retinoid cycle proteins, RPE65 and CRALBP, were also reduced in Pcdh15R250X mice, indicating a dual role for protocadherin-15 in photoreceptors and RPE. Exogenous 9-cis retinal improved ERG amplitudes in Pcdh15R250X mice, suggesting a basis for a clinical trial of FDA approved retinoids to preserve vision in USH1F patients.
Usher syndrome (USH) is estimated to be responsible for more than 50% of deaf-blind cases, 8-33% of patients with RP and 3-6% of congenitally deaf individuals (Boughman et al., 1983; Brownstein et al., 2004b; Vernon, 1969). Clinical data review studies estimated a prevalence of 3.2 to 6.2 per 100,000 for USH cases (Boughman & Fishman, 1983; Koenekoop et al., 1993). However, a molecular diagnosis study in children with hearing loss found variants in USH-associated genes in 11% and estimated a frequency of 1/6000 individuals afflicted with USH in the USA (Kimberling et al., 2010). Assuming similar prevalence, this would translate into 255,000 to 1.34 million USH cases worldwide. However, this estimate varies considerably in specific population substructures. For instance, the p.Arg245* founder variant of PCDH15 (USH1F) has ~2% carrier frequency amongst Ashkenazi Jews accounts for nearly 60% of their USH1 cases (Ben-Yosef et al., 2003). Thus, we speculate that a comprehensive understanding of the pathophysiology and disease mechanisms is a prelude for developing therapeutic interventions for Usher syndrome after clinical trials.

Loss of vision in individuals with USH1, an autosomal recessive disorder, begins towards the end of their first decade of life due to retinitis pigmentosa (RP), eventually leading to near total blindness. Night blindness is an early sign in USH1 subjects followed by constriction of the visual field (tunnel vision) and finally clinical blindness (Vernon, 1969). Characteristic fundus features include pigmentary retinopathy, narrowing of the retinal vessels and a pale appearance of the optic disk (Toms et al., 2020). Vestibular dysfunction in USH1 manifests as a delay in development of independent ambulation while hearing loss is usually severe to profound, congenital and sensorineural (Ahmed et al., 2003b; Smith et al., 1994). Cochlear implants can restore auditory perception in USH1 patients (Brownstein et al., 2004b; Pennings et al., 2006).
but presently there is no effective treatment for the vision loss due to RP. Moreover, there is a lack of longitudinal data for the natural history of ocular abnormalities associated with variants of PCDH15 in humans. Only anecdotal clinical data has been reported thus far (Ahmed et al., 2001a; Ben-Yosef et al., 2003; Brownstein et al., 2004a; Jacobson et al., 2008). Here, we describe the natural history of retinopathy in 13 individuals followed for up to 30 years with an Ashkenazi Jewish recessive founder variant of PCDH15. Eleven patients from nine families were homozygous for the p.Arg245*, leading to truncation of the encoded protein, protocadherin-15. Two additional patients had compound heterozygous genotypes that included one p.Arg245* allele.

Protocadherin-15 is a member of a large cadherin superfamily of calcium-dependent cell–cell adhesion molecules (Ahmed et al., 2006a; Ahmed et al., 2001b; van Roy, 2014). Within the vertebrate inner ear, protocadherin-15 is required for the structural maintenance and the mechanotransduction function of the sensory hair cells (Ahmed et al., 2006b; Kazmierczak et al., 2007). In the retina, protocadherin-15 is localized to the outer limiting membrane of photoreceptors (PR) and in Müller glia (Reiners et al., 2005b; van Wijk et al., 2006). We previously reported a reduction of ERG a- and b-waves amplitudes (~40%) at 5 weeks of age in at least two Pcdh15 alleles in mice (Pcdh15av-5J and Pcdh15av-jfb) (Haywood-Watson et al., 2006b). However, the exact molecular function of protocadherin-15 in the retina remains elusive. Here, we describe the pathophysiology and function of protocadherin-15 in the retina of a novel murine model. Finally, electroretinogram (ERG) data show significant improvement after treatment of this USH mouse model with 9-cis retinal, raising the possibility that exogenous retinoids could preserve vision in USH1F patients.
Results and Discussion

Spectrum and longitudinal ocular phenotypic data revealed early onset rod-cone dystrophy in USH1 subjects

We reviewed the medical records of 13 patients enrolled in an Institutional Review Board-approved protocol to study Usher syndrome. Subsequent to congenital profound deafness, the first reported symptom was difficulty with vision at night. Ophthalmic manifestations depended on the age of the patient and the stage of retinal degeneration (Table 1).

Electroretinography recordings were at noise-level for both scotopic and photopic responses, suggesting dysfunctional photoreceptors (figure1-figure supplement 1). In young patients with early stages of the retinal degeneration, findings included mottling of the retinal pigment epithelium with early pigment redistribution, and mild to moderate vascular attenuation with typically preserved macular reflexes (Figure 1a). As RP progressed, more extensive pigment abnormalities were observed with deposition of bone spicules, severe attenuation of the retinal vasculature, macular atrophic changes, and a waxy pallor of the optic nerve head (Figure 1b). In advanced stages these changes became more prominent and widely distributed throughout the fundus (Figure 1c, figure1-figure supplement 1). Cataracts were common, especially posterior subcapsular opacities. The panels in Figure 1d show the progression of macular atrophic changes over a 12-year period in a patient with compound heterozygous p.Arg245*/p.Arg929* variants. Both of these alleles of PCDH15 are predicted to cause truncation of the protocadherin-15 protein. Kinetic visual field testing (Table 1) showed early loss of the ability to detect the smaller and dimmer target (I1e) in all but one patient (LMG268 #1722 at age 37 years). Early midperipheral scotomas and severe constriction were noted while testing the I4e isopter (target is equal in size to I1e but brighter). Progressive constriction of the V4e visual field isopter (largest
and brightest target) is seen in Figure 1e where the horizontal diameter is binned by the decade of life. Figure 1f shows an increase in logMAR visual acuity, corresponding to a decline in Snellen best-corrected visual acuity (BCVA), starting at the fourth decade of life. Kaplan-Meier survival curves (Figure 1g) with parameters corresponding to legal blindness (acuity at 20/200 and visual field limited to 20 degrees) demonstrate severe visual function loss by the fifth decade.

**Pcdh15R250X knockin mutant recapitulates human p.Arg245* Usher phenotype**

In order to investigate the precise role of protocadherin-15 in light transduction and the mechanism of visual deficits observed in patients homozygous or compound heterozygous for the recessive Arg245* pathogenic variant of *PCDH15*, we used CRISPR/Cas9 technology to engineer a mouse model with the *Pcdh15R250X* variant (Methods) (Cong et al., 2013). Immunostaining showed that protocadherin-15 is localized to the inner segments of the PR, the outer plexiform layer, and the ganglion cell layer as reported previously (Haywood-Watson et al., 2006a). However, the RPE was not assessed for the localization of protocadherin in those studies. Using immunohistochemistry, here we show that protocadherin-15 is also expressed in the RPE (Figure 2-figure supplements 1). The p.Arg250* variant is located in an exon common to all *Pcdh15* transcripts and, consequently, is predicted to cause a complete loss of all known protocadherin-15 isoforms (Ahmed et al., 2006b). Indeed, with immunostaining, we could not detect protocadherin-15 expression in retinal tissue (Figure 2-figure supplement 1a-b) or cochlear tissue (Figure 2-figure supplement 1c) from *Pcdh15R250X/R250X* mutant mice.

Consistent with previously published *Pcdh15* mouse models (Alagramam et al., 2011; Haywood-Watson et al., 2006a; Senften et al., 2006) we detected no auditory-evoked brainstem
responses (ABRs) in mutant Pcdh15\[^{R250X}\] mice at P16, the earliest postnatal day that ABRs can be reliably detected (Figure 2-figure supplement 2a), indicating that they were profoundly deaf. Furthermore, Pcdh15\[^{R250X}\] mutant mice displayed abnormal motor vestibular behaviors such as circling, hyperactivity, and head bobbing. Behavioral tests including exploratory-behavior and tail-hanging tests confirmed that these deaf mice also have a significant vestibular dysfunction (Figure 2-figure supplement 2b, c). Lastly the Pcdh15\[^{R250X}\] mutant cochlear and vestibular hair cells also had no functional mechanotransduction (Figure 2-figure supplement 2d), accounting for deafness, and at P60 also showed degeneration of hair cells in the organ of Corti (Figure 2-figure supplement 3). Taken together, our data indicate that the Pcdh15\[^{R250X}\] mutants recapitulates human p.Arg245* deafness and peripheral vestibular areflexia.

To parallel the visual examinations performed in patients, we assessed the visual function of Pcdh15\[^{R250X}\] mutant mice using full-field electroretinography (ERG). Dark-adapted (scotopic) ERG waves, which are preferentially driven by rod photoreceptors at low light intensity and by rod and cone photoreceptors at high light intensity, showed normal wave architecture albeit with reduced amplitudes (Figure 2-figure supplement 4a). Quantification showed significant reduction in amplitudes of the a-wave derived primarily from the photoreceptor layer and the b-wave derived from Müller glia and bipolar neurons in one-month-old Pcdh15\[^{R250X}\] mutant mice as compared to littermate control mice (Figure 2a). Similarly, photopic ERG amplitudes, primarily representing cone-mediated function, were also reduced in one-month-old Pcdh15\[^{R250X}\] mutant mice (Figure 2b). The b- to a-wave ratio (b/a) was similar across genotypes indicating that deficits were manifested mainly at the photoreceptor level (Figure 2-figure supplement 4b). We then performed ERGs at 2-3, 6-7, and 12-14 months of age. Pcdh15\[^{R250X}\] mutant mice consistently had lower scotopic and photopic ERG amplitudes compared to controls (Figure 2c-
indicating that the functional deficits observed at one month were not due to delayed development. Further, to assess the visual cycle dysfunction and dark adaptation, we assessed the recovery of a-wave amplitude following bleaching of more than 90% of rhodopsin (Kolesnikov et al, 2020). We observed equivalent recovery of a-wave irrespective of genotype (Figure 2-figure supplement 4c), but we did observe that initial single flash a-wave amplitude was lower in mutant mice (Figure 2-figure supplement 4d), which suggests functional alterations in phototransduction such as are indicated by aberrant light-dependent translocation of arrestin and transducin (see details below). To correlate functional deficits with structural integrity of the retina, we performed non-invasive in vivo retinal imaging using optical coherence tomography (OCT) in young (1-2 months) and old (12-14 months) mice, which showed no gross structural abnormality in homozygous Pcdh15^{R250X} mutants (Figure 2i). However, we did note a small but significant decrease in the ONL thickness in 12-14 months old mutant mice (Figure 2j).

Mechanisms contributing to ERG defects due to protocadherin absence

We hypothesized that the functional deficits, reflected by abnormal ERG findings, without structural impairment of the retina might result from deficits in the phototransduction cascade or the visual retinoid cycle. The phototransduction cascade mediates the transduction of light into neuronal signals, while the visual retinoid cycle regenerates a key chromophore, 11-cis retinal. The rod outer segments (OS) are exquisitely adapted for light transduction. Phototransduction proteins are generated in the photoreceptor cell body and delivered to the OS via the inner segment (IS) and connecting cilium. Under photopic conditions (daylight), arrestin translocates from IS of the photoreceptors to the OS, to desensitize the opsin. Conversely, transducin translocates from OS to IS of the photoreceptors allowing arrestin to bind to opsin.
We found significant mislocalization of both arrestin and transducin to the photoreceptor IS and OS in Pcdh15<sup>R250X</sup> mutant mice under light-adapted conditions, whereas transducin was correctly localized only to the IS and arrestin only to the OS in control mouse retinas (Figure 3a, Figure 3-figure supplements 1). In dark adapted conditions, arrestin was correctly localized to the IS and transducin to the OS in both mutant and control mice (Figure 3-figure supplements 1a, c-d). Finally, opsin was correctly localized only in the OS under both dark- and light-adapted conditions (Figure 3-figure supplements 1b, e), indicating that protocadherin-15 is essential for rapid shuttling of proteins from IS to OS and vice-versa in response to adaptation to light.

The 11-cis retinal complexes with opsin to form rhodopsin. Absorption of a single photon by 11-cis retinal leads to its photo-isomerization to all-trans retinal within femtoseconds (Nogly et al., 2018), thus activating opsin and initiating the phototransduction cascade. Consequently, there is decoupling of opsin and all-trans retinal. All-trans retinal must be re-isomerized to 11-cis retinal to form rhodopsin again. These enzymatic steps occur in the retinal pigment epithelium (RPE) (Saari, 2000; Travis et al., 2007; Wald & Brown, 1956). Next, we assessed the levels of crucial retinoid cycle proteins such as RPE65, an essential isomerase which catalyzes the conversion of all-trans retinyl ester to 11-cis retinol (Jin et al., 2005; Moiseyev et al., 2005; Redmond et al., 2005), CRALBP (cellular retinaldehyde-binding protein), a key retinoid transporter, and IRBP (interphotoreceptor retinoid binding protein). These studies were rationalized based on the findings that protocadherin-15 is a binding partner of myosin VIIA (Senften et al., 2006), which also interacts with RPE65 (Lopes et al., 2011). Similar to protocadherin-15, pathogenic variants of myosin VIIA also causes USH1 (Jacobson et al., 2011;
Intriguingly, immunoblotting revealed significantly reduced quantities of RPE65 and CRALBP, but not IRBP in Pcdh15<sup>R250X</sup> mutant mice (Figure 3b, c).

We also quantified the absolute retinoid levels within the eyes after overnight dark adaptation, and as compared to controls found reduced levels of retinoids, particularly 11-cis-retinaloxime, in Pcdh15<sup>R250X</sup> mutant mice (Figure 3d). 11-cis retinaloxime levels correlate with photoreceptor rhodopsin levels. Next, we quantified the retinoids levels one hour after dark adaptation following bleaching with 15,000 lux for one hour (Li <i>et al</i>, 2019). Reduction of 11-cis retinaloxime in control and mutant mice retinae (Figure 3d) correlated with prebleach levels, as did increase in all-trans retinyl esters. These findings from Pcdh15<sup>R250X</sup> mutants suggest a reduced function of the visual cycle due to reduced expression of RPE65 and CRALBP. Since we observed lower visual cycle proteins (RPE65 and CRALBP), we also assessed the structure of the RPE, the main cell type harboring the key enzymes of the visual cycle. Transmission electron microscopy showed no gross structural deficits in the RPE (Figure 3-figure supplement 2). Together, our data indicates that the loss of protocadherin-15 in the retina leads to aberrant translocation of proteins involved in the phototransduction cascade and reduced levels of key retinoids and enzymes involved in the visual retinoid cycle.

**Pre-clinical administration of exogenous retinoids**

We hypothesized that low levels of retinoids in the mutant mice could be overcome by providing exogenous retinoids, thus rescuing the ERG deficits (Palczewski, 2010; Sethna <i>et al</i>, 2020). To test this hypothesis, we first performed baseline ERGs on 2-3-month-old control and Pcdh15<sup>R250X</sup> mutant mice. One week later, Pcdh15<sup>R250X</sup> mutant mice were injected intraperitoneally (IP) with 9-cis retinal, an analog of naturally occurring 11-cis retinal. Control
mice were injected with vehicle. ERGs were performed the next day after overnight dark adaptation. Remarkably, a single treatment of Pcdh15R250X mutant mice with 9-cis retinal was sufficient to increase their ERG amplitudes to levels comparable to those in vehicle-injected wild type controls (Figure 4a-b, Figure 4-figure supplement 1). Similarly, we also observed an improvement in cone function to levels of the vehicle injected control mice (Figure 4d). The b-to a-wave ratio was consistent with vehicle injected control mice or baseline Pcdh15R250X mutant mice (Figure 4c), suggesting a proportional increase in photoreceptor function after retinoid therapy. To confirm the 9-cis retinal delivery and metabolism, in a separate cohort of mice, 24 hours post-injection followed by 2-hour light exposure, we evaluated the retinaloxime levels, including 9-cis retinaloxime, both in the liver and retina. As expected, we found trace levels of 9-cis retinaloxime levels in the liver and retinae (Figure 4-figure supplement 1).

Next, to assess the impact of exogenous retinoids in aged animals, we performed similar experiments using 6-7-month-old mice. We found a comparable increase in functional activity with a single IP injection of 9-cis retinal in mutant mice as compared to the same cohort of mutant mice assessed one week earlier (baseline Pcdh15R250X mutant mice). The ERG amplitudes of 9-cis retinal-injected mutant mice were nearly indistinguishable from those of vehicle-injected wild type control mice (Figure 4d-f). Finally, in a separate cohort of 6-7-month-old mutant mice we assessed the longevity of the retinoid-mediated improvement. We found that by two weeks after 9-cis retinal treatment, the impact of exogenous retinoid treatment on ERG improvements were reduced (Figure 4g).

Next, we assessed whether exogenous 9-cis retinal treatment also improved translocation of arrestin and transducin in Pcdh15R250X mutant mice. For these studies, we injected a cohort of mutant mice with either 9-cis retinal or vehicle. Overnight dark-adapted mice were exposed to
normal room light for two hours and their retinas examined for localization of arrestin and transducin. We found mis-localization of arrestin and transducin in mutant mice injected with 9-cis retinal as well (Figure 4-figure supplement 2). Taken together, our data link the visual deficits to the retinoid cycle dysfunction in Pcdh15<sup>R250X</sup> mutant mice and provides a starting point to investigate the possibility of therapeutically boosting visual function in USH1F patients.

The spectrum and longitudinal ophthalmic phenotypes of USH1F patients homozygous for the p.Arg245<sup>*</sup> variant (or compound heterozygotes) consisted of a rod-cone dystrophy and are relatively uniform across this cohort of patients. They include an onset of symptoms such as night vision difficulties and visual field deficits in the first or early second decade, the presence of macular atrophy with reduction in central visual acuity by the third decade and, subsequently, the progressive constriction of visual fields resulting in tunnel vision between the third and fifth decades of life. Progressive posterior subcapsular cataract and optic nerve head atrophy are also frequent manifestations of this PCDH15 genotype.

Pcdh15<sup>R250X</sup> mutant mice have a retinal dysfunction as early as one month after birth. We do not observe gross retinal damage, however in aged mutant mice we do observe marginal loss of ONL thickness. Our data indicates that protocadherin-15 has a dual role in photoreceptors and the RPE. First, at the junction of the photoreceptor IS and OS, where protocadherin-15 is localized (Reiners <i>et al</i>, 2005a), the loss of protocadherin-15 leads to disrupted shuttling of arrestin and transducin under light-adapted conditions. Second, within the RPE, loss of protocadherin-15 is associated with lower levels of two key visual retinoid cycle enzymes, CRALBP and RPE65. Reduced levels of RPE65 were reported in Myo7a knockout mice (Lopes <i>et al</i>, 2011), a binding partner of protocadherin-15. Further, CRALBP facilitates the transport of 11-cis retinal between the RPE and the photoreceptor OS (Saari <i>et al</i>, 2001). Hence, reduced
levels of RPE65 and CRALBP lead to delayed and reduced regeneration and transport of 11-cis retinal to the photoreceptor OS and thus we observed a concordant reduction in levels of 11-cis retinal oxime. Our data provide a plausible explanation for reduced ERG amplitudes without gross retinal degeneration in Pcdh15<sup>R250X</sup> mutants, suggesting this may also be the case for other Pcdh15 mutant mice (Haywood-Watson et al., 2006a; Libby & Steel, 2001; Liu et al., 2007; Peng et al., 2011).

Unlike the typical human ocular manifestations of USH1, which have severe retinal degeneration, our mouse model has much less severe pathophysiology. The discordance between retinal pathologies in humans and mice may be further attributable to the structural differences in their photoreceptors, particularly the presence of calyceal process in humans, monkeys and frog, but not in rodents (Sahly et al., 2012), light exposure (Lopes et al., 2011) or environmental factors. The role of Usher proteins in the calyceal processes is supported by recent observations of photoreceptor degeneration in Ush1 frog models that have calyceal processes (Schietroma et al., 2017). Further, this is consistent with reported ocular phenotypes of other USH mouse models on C57BL/6J background (Haywood-Watson et al., 2006a; Jacobson et al., 2008; Liu et al., 2007; Liu et al., 1999; Williams et al., 2009). However, a recent study showed degeneration of cone photoreceptors in Ush1c and Ush1g knockout mice on an albino background (Trouillet et al., 2018), which suggest that pigmentation might be providing protection to mice against ambient light condition in their housing facilities. Currently, we are backcrossing Pcdh15<sup>R250X</sup> to generate congenic mice with an albino background. Future studies will assess the photoreceptor fate and ERG progression in these mice.

In conclusion, documenting the natural history and the degree of clinical variability of the ocular phenotype in human and animal models is pivotal for evaluating efficacy and potential
therapeutics in future clinical trials. Our longitudinal USH1F patient ocular data show that

significant vision and photoreceptors are preserved until the third decade of life, providing a long

window of opportunity. Our results with an 11-cis retinal analog, 9-cis retinal, raise the

possibility that longer lasting analogs such as 9-cis retinyl acetate, which has an excellent safety

profile (Koenekoop et al, 2014; Scholl et al, 2015) or a synthetic version of 11-cis β-carotene,

whose capsule formulation is already approved by the United States Food and Drug

Administration (Rotenstreich et al, 2013), could preserve vision in USH1F Usher syndrome

patients. Furthermore, in mouse models lacking key visual cycle enzyme RPE65 or with one of

the most common variants of opsin causing RP (p.Pro23His), administration of retinoids has

been shown to preserve photoreceptor morphology or proper folding of opsin to a greater extent

(Maeda et al, 2009; Noorwez et al, 2004), and thus might also extend the life of functional

photoreceptors in USH1 patients. Based on our pre-clinical data in mouse and prior human trials,
a clinical trial in USH1F patients may show benefit if the retinoid is administered early in life.


Material and Methods

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**Patient assessment**

The records of 13 patients were reviewed under the National Eye Institute, National Institutes of Health protocol 08-EI-N014. Informed consents were obtained from the patients to conduct this research. Eleven were homozygous for the p.Arg245* founder variant was associated with the majority of Usher syndrome type 1 of the Ashkenazi Jews in our study and 2 subjects who were compound heterozygous with one p.Arg245* variant and a second pathogenic variant of PCDH15 in trans. Data included demographic information, age of onset of visual symptoms, date of ophthalmic exams and reason for visit, best corrected visual acuity (BCVA), visual fields, presence and type of lenticular opacities, fundus exam findings, Optical Coherence Retinography when available and electroretinography. The horizontal diameter of the V4e, I4e, and I1e isopters on Goldmann Visual Fields were measured. Five patients were seen at the NIH Clinical Center under protocol 05-EI-0096 and three had follow-up visits over a thirty-year period. These patients underwent a complete phathalmologic examination including best-corrected visual acuity (BCVA) with manifest refraction, biomicroscopy and photography of lens opacity, if present, and visual field evaluation by Goldmann kinetic perimetry (GVF). Dilated
ophthalmoscopic examination was performed after instillation of phenylephrine 2.5% and tropicamide 1%. Digital photography of the retinal fundus was performed. Snellen visual acuity was measured using ETDRS charts. In patients whose visual acuity was reduced to a degree preventing them from reading the chart, the ability to recognize hand motion (HM) or perceive light (LP) was documented. The presence or absence of cystoid macular changes and/or atrophic pigmentary macular changes were assessed by ophthalmoscopy and/or macular photography.

Generating *Pcdh15*<sup>R250X</sup> mice

*Pcdh15*<sup>R250X</sup> mice were generated by the Cincinnati Children’s Hospital Genetics Core using CRISPR/Cas9 technology and then transferred to the University of Maryland School of Medicine (UMSOM) facilities. In addition to the desired mutation (in red, see below) that changes the codon of R250 from CGA to TGA, silent mutations (in green) were introduced to create a Hae II restriction endonuclease site (underlined) which is used to facilitate genotyping as well as to prevent recutting by Cas9 nuclease.

PAM

WT: …GACCGTGCAAAAAATCTGAATGAG<sup>AGG</sup>C<sup>G</sup>AACAACCACCA…

R

X

KI: …GACCGTGCAAAAAATCTGAATGAG<sup>cGc</sup>Gc<sup>G</sup>AACAACCACCA…

Hae II

Heterozygous founder mice were bred with wild type (WT) C57BL/6J mice and the colony was further expanded on the C57BL/6J background. Mice are genotyped using primers VS4576:

TTCACCTTCCATTCCCCCAAC and VS4577: CTTACC GGAGTCTCAGTTTCAGG, which
generates a 343 bp amplicon that was also Sanger sequenced. Mice were housed in a facility with 12 hours of light and 12 hours with the lights off. Mice were fed after weaning on a standard mouse diet and with water available ad libitum. We followed the ARRIVE guidelines for reporting animal research and studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal procedures were approved by the UMSOM IACUC (Institutional Animal Care and Use Committees).

**Electroretinography (ERG) and Optical Coherence Tomography (OCT)**

ERGs were recorded as previously described (Sethna *et al.*, 2016). Overnight dark-adapted mice were anesthetized with a combination of ketamine-xylazine (100 mg/kg and 10 mg/kg, respectively), followed by dilation of pupils with 1% Tropicamide. A gold loop wire electrode was placed on the cornea, a reference electrode was placed on the scalp under the skin and a ground electrode was placed under the skin near the tail. ERG waveforms were acquired using sequentially brighter stimuli (0.003962233 to 3.147314 cd x s/m²) with 5-60 sec intervals using the Diagnosys ColorDome Ganzfeld system (Diagnosys systems, Lowell, MA). Three to five waveforms per intensity were averaged. Photopic, cone-only, responses were acquired at a single bright flash (3.15 cd x s/m²) under a steady rod-suppressing field of 30 cd x s/m², with 10 waves averaged. Waves were analyzed using inbuilt Espion software. For exogenous 9-*cis* retinal treatment, animals received intraperitoneal 0.25 mg 9-*cis* retinal (Sigma Aldrich Inc., Saint Louis, MO) (25 mg dissolved in 200 µl 100% ethanol) and diluted 1:10 in vehicle (180 µl sterile filtered 10% BSA in 0.9% NaCl solution) or vehicle only (20 µl 100% ethanol and 180 µl 10% BSA in 0.9% NaCl solution), in the dark (Sethna *et al.*, 2020; Xue *et al*). Animals were dark
adapted overnight and ERGs were performed as above. OCT was performed using Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). Mice were anesthetized and dilated as above. A custom designed plano-concave contact lens micro-M 2.00/5.00 (Cantor & Nissel Ltd, Northamptonshire, UK) was used to obtain cross sections of the entire retina. Outer nuclear quantification was performed as detailed before (Zeng et al, 2016).

**Immunohistochemistry (eye and ear) and FM1-43 uptake**

Mice (1 to 3-month-old) were dark adapted overnight and euthanized before light onset and eyes were enucleated following CO₂ asphyxiatiion followed by cervical dislocation or exposed to normal room light for 2 hours after light onset and euthanized as above and processed as below. Dark adapted procedures were performed under very dim red light. Eyes were immediately fixed in Prefer fixative (Anatech LTD, Battle Creek, MI), paraffin embedded and stained using standard protocols (Sethna et al., 2016; Sethna & Finnemann, 2013). Briefly, 7 µm sections were deparaffinized, rehydrated in PBS, blocked and permeabilized with 10% normal goat serum/0.3% triton-X 100 for 2 hours at RT, and incubated overnight at 4°C with the indicated primary antibodies to transducin (1:100 dilution, #Sc-517057, Santa Cruz Biotechnology, Dallas, TX) and arrestin (clone C10C10, 1:25 dilution, kind gift from Drs. Paul Hargrave and Clay Smith, University of Florida, FA). The following day, sections were incubated with Alexa fluor labeled goat secondary antibodies (1:250) and DAPI (Thermo Fisher Scientific, Waltham, MA) to label nuclei. Sections were scanned using the UMSOM core facility Nikon W1 spinning disk microscope and images were processed using FIJI software (Schindelin et al, 2012). To stain for protocadherin-15, dissected eyes were fixed in 4% PFA (Electron Microscopy Sciences,
Hatfield, PA) and processed as above using a previously described custom antibody targeting the C-terminus of protocadherin-15 (PB303; 1:200) (Ahmed et al., 2003a).

P60 temporal bones were fixed and processed for immunocytochemistry as previously described (Riazuddin et al., 2012). The cochlear and vestibular sensory epithelia were isolated, fine dissected and permeabilized in 0.25% Triton X-100 for 1 hour and blocked with 10% normal goat serum in PBS for 1 hour. Tissue samples were probed overnight with antibodies against myosin VIIa or custom antibody targeting the N-terminus of antibody protocadherin-15 (HL5164; 1:200 dilution)(Ahmed et al., 2006b), and after three washes, were incubated with the secondary antibody for 45 mins at room temperature. Rhodamine phalloidin was used at a 1:250 dilution for F-actin labeling. All images were acquired using a LSM 700 laser scanning confocal microscope (Zeiss, Germany) using a 63×1.4 NA or 100×1.4 NA oil immersion objectives.

Stacks of confocal images were acquired with a Z step of 0.5µm and processed using ImageJ software (National Institutes of Health, Bethesda, MD).

Cochlear and vestibular explants were dissected at postnatal day 0 (P0) and cultured in a glass-bottom petri dish (MatTek, Ashland, MA). They were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA) for 2 days at 37°C and 5% CO2. Explants were incubated for 10 sec with 3 µM FM1-43, washed three times with Hank's balanced salt solution, and imaged live using a Zeiss LSM 700 scanning confocal microscope.

### Auditory Brainstem Response (ABR) measurements

Hearing thresholds of heterozygous and homozygous *Pcdh15*R250X mice at P16 (n = 5 each genotype) were evaluated by recording ABRs. All ABR recordings, including broadband clicks
and tone-burst stimuli at three frequencies (8, 16, and 32 kHz), were performed using an auditory-evoked potential RZ6-based auditory workstation (Tucker-Davis Technologies Alachua, FL) with a high frequency transducer. Maximum sound intensity tested was 100 dB SPL. TDT system III hardware and BioSigRZ software (Tucker Davis Technology, Alachua, FL) were used for stimulus presentation and response averaging.

Vestibular testing
Exploratory tests were performed as previously described (Michel et al., 2017). Briefly, mice were placed individually in a new cage. A camera was placed on top of the cage to record movements of mice for 2 mins and tracked using ImageJ software. Tail hanging tests were performed as follow: Mice were held five centimeters above a tabletop. The test scores were given as following: normal behavior was demonstrated by a “reaching position”, with a score of 4, by the extension of limb and head forward and downward aiming to the tabletop. Mice with abnormal behavior, ranked with a score of 1, tried to climb towards the examiner’s hand, curling the body upward reaching with the head to the tail one time. Mice ranked with a score of 0, tried to climb towards the examiner’s hand, curling the body upward reaching with the head to the tail multiple times.

Retinoid extraction and analysis
All procedures for retinoid extraction were performed under red safelights. Overnight dark-adapted mice were euthanized with CO₂, eyes enucleated, lens and vitreous removed, followed by freezing the eyecups in pairs on dry ice. These were stored at -80°C until retinoid extraction was performed. Mouse eyecup pairs were homogenized in fresh hydroxylamine buffer (1 ml of
0.1 M MOPS, 0.1 M NH₂OH, pH 6.5). 1 ml ethanol was added, samples were mixed and incubated (30 min in the dark at RT). Retinoids were extracted into hexane (2 X 4 ml), followed by solvent evaporation using a gentle stream of argon at 37°C. After reconstituting in 50 μl mobile phase, the retinoid samples were separated on LiChrospher Si-60 (5 μm) normal-phase columns (two 2.1 x 250 mm in series; ES Industries, West Berlin, NJ) using an H-Class Acquity UPLC (Waters Corp., Milford, MA) along with standards at a flow rate of 0.6 ml/min, following published methods (Landers & Olson, 1988). Retinaloxime standards were prepared from 9-cis-retinal (Toronto Research Chemicals, Toronto, Canada), 11-cis-retinal, National Eye Institute, NIH), and all-trans-retinal (Sigma Aldrich, Saint Louis, MO) using published methods (Garwin & Saari, 2000). Also, synthetic retinyl palmitate (Sigma Aldrich Inc., St. Louis, MO) was used as a standard. Absorbance was monitored at 350 nm for retinaloximes and at 325 nm for retinyl esters. Peak areas were integrated and quantified using external calibration curves. Data were analyzed using Empower 3 software (Waters Corp., Milford, MA).

**Data analysis**

Four to eight animals per time point/genotype/treatment for ERG analysis were used. One-way ANOVA with Tukey’s post-hoc test or Student’s t-test was used to compare control sample to test samples with the data presented as mean ± SEM. Differences with p < 0.05 were considered significant. Data were analyzed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

**Contact for reagent and resource sharing**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Zubair M. Ahmed, PhD (ZMAhmed@som.umaryland.edu).
Competing financial interests

All authors declare no competing financial interests.

Acknowledgments

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Table 1: Ophthalmic clinical manifestations of patients with biallelic PCDH15 mutations

<table>
<thead>
<tr>
<th>LMG210 #1563</th>
<th>Age (yrs.)</th>
<th>BCVA OD : OS</th>
<th>Visual Field OD</th>
<th>Visual Field OS</th>
<th>Lens</th>
<th>Macula</th>
<th>Spicules / Mottling</th>
<th>Optic Nerve</th>
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<td>90, ND, ND</td>
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<td>Pale</td>
<td>N/A</td>
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<td>Mild PSC</td>
<td>Pigment</td>
<td>Spicules</td>
<td>Pale +3</td>
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<tr>
<td>35</td>
<td>HM; HM</td>
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<td>Normal</td>
<td>Spicules</td>
<td>Pale</td>
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<td>15, 2, 0</td>
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<td>Atrophy OD</td>
<td>Spicules</td>
<td>Pale</td>
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<td>Spicules</td>
<td>Pale</td>
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<td>ND</td>
<td>PSC + NS OD; IOL OS</td>
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<td>Pale</td>
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<td>ND</td>
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<td>CME</td>
<td>Spicules</td>
<td>ONH Swelling Resolved Swelling</td>
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<td>CME</td>
<td>Spicules</td>
<td>Pale +1</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Pale +1</td>
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<td>Clear</td>
<td>Atrophy</td>
<td>Spicules</td>
<td>Pale +1</td>
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<td>HVF</td>
<td>HVF</td>
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<td>Pale</td>
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<td>55</td>
<td>20/400; 20/300</td>
<td>3, 0, 0</td>
<td>5, 0, 0</td>
<td>IOL OU</td>
<td>Atrophy</td>
<td>Spicules</td>
<td>Pale +3</td>
</tr>
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<td>LMG322 #1917</td>
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<td>Spicules</td>
<td>Normal</td>
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<td>Spicules</td>
<td>Normal</td>
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<td>Spicules</td>
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<td>LMG125 #1221</td>
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<td>CME, ERM</td>
<td>Spicules</td>
<td>Pale</td>
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<td>Normal</td>
<td>Spicules</td>
<td>Pale +1</td>
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<tr>
<td>38</td>
<td>20/60, 20/60</td>
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<td>60, 4, 0</td>
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<td>15, 0, 0</td>
<td>22, 0, 0</td>
<td>PSC</td>
<td>Atrophy</td>
<td>Spicules</td>
<td>Pale +3</td>
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<td>ERM</td>
<td>RPE atrophy</td>
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<td>Clear</td>
<td>ERM</td>
<td>Spicules</td>
<td>Pale +3</td>
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<tr>
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<td>30, 4, 0</td>
<td>PSC +1</td>
<td>Atrophy</td>
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<td>Pale +3</td>
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</table>
11 patients in this study are homozygous for the p.Arg245* variants while two siblings carry compound heterozygous variants, p.Arg245*/p.Arg929*. Visual acuity assessments consistently show a decline between the third and fourth decade of life. Visual field loss in these subjects, shown in Table 1, further support severe retinitis pigmentosa. Macular atrophy and PSC cataract appear early and may contribute to the observed reduction in visual acuity. Optic nerve head pallor is also frequent and advanced. Normal Goldmann Visual Field Perimetry horizontal diameters are in the range of: V4e – 150 to 160 degrees, I4e 130 to 140 degrees, and I1e 20 to 30 degrees. BCVA: Best Corrected Visual Acuity, OD: right eye, OS: left eye, HM: Hand Motion visual acuity, LP: Light Perception, HVF: Humphrey Visual Field, PSC: Posterior Subcapsular Cataract, NS: Nuclear Sclerosis Cataract, IOL: Intraocular lens, CME: Cystoid Macular Edema, ERM: Epiretinal Membrane, RPE: Retinal Pigment Epithelium, ONH: Optic Nerve Head, ND: Not Done, N/A: Not Available
Figure 1: USH1F p.Arg245* spectrum and longitudinal eye phenotype

a-c Fundus images depicting the spectrum of retinal findings in p.Arg245* USH1F patients, show mottling of pigment epithelium, attenuation of retinal vasculature and pallor of optic nerve head seen in all three fundus photos. Peripheral bony spicules and macular atrophy are noted b. Diffuse atrophy and advanced retinal degeneration are seen in c. d Longitudinal progression of macular atrophic changes (arrowheads point at edge of macular atrophic area) over a 12-year period in a USH1F patient who is compound heterozygous for p.Arg245*/p.Arg929*. e Mean and SEM of Goldmann visual field diameters for patients with data binned by decade of life. f Mean and SEM of best corrected visual acuity binned by decade of life for all patient visits. g Survival analysis curves for visual acuity (logMAR visual acuity >1 i.e. acuity worse than 20/200) and visual field (visual field < 20 degrees in better eye). These values were chosen since they usually denote visual function at legal blindness levels. SEM, standard error of the mean.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Source data 1. Excel sheet showing data from clinical investigations plotted in Figure 1e, f, g.

Figure supplement 1. Fundus autofluorescence, Optical Coherence Tomography, and electroretinography in compound heterozygous patient LMG197#1831.

Figure 2: Loss of protocadherin-15 leads to visual dysfunction over a period of 1 year

a Quantification of scotopic (dark adapted) responses from littermate control (Pcdh15+/+ or Pcdh15R250X/+ ) and mutant (Pcdh15R250X/R250X) mice at one-month of age revealed progressive loss of both a- (left panels) and b-wave (right panels) amplitudes in mutant mice. Representative ERG waveforms are shown in Extended data 3b. b Quantification of photopic (light adapted) b-wave indicates decline of cone photoreceptor function in mutant mice. c-h Quantification of
scotopic ERG amplitudes c, e, g and photopic ERG amplitudes d, f, h at indicated ages shows sustained decline in amplitudes over time in Pcdh15 mutant mice. i Representative OCT images from mice of denoted genotype, shows no gross retinal degeneration in young (1-2 months, top panels) or old (12-14 months, bottom panels) mice. j Quantification of outer nuclear layer (ONL) of images showed in i, shows mild loss of ONL in aged mutant mice. Data presented as mean ± SEM. Each data point represents an individual mouse. Data presented as mean ± SEM. Student unpaired t-test, p<0.05 (*), <0.01 (**).

The online version of this article includes the following source data and figure supplement(s) for figure 2:

Source data 1. Excel sheet showing data from experiments plotted in Figure 2.

Figure supplement 1. Validation of loss of protocadherin-15 in the retina and cochlea of Pcdh15R250X mutant mice.

Figure supplement 2. Pcdh15R250X mutant mice have profound hearing loss and severe vestibular system dysfunction

Figure supplement 2- source data 1. Excel sheet showing data from experiments plotted in Figure 2-figure supplement 2a, b, c.

Figure supplement 3. Pcdh15R250X mutant mice have degeneration of sensory hair cells in the organ of Corti.

Figure supplement 4. Loss of protocadherin-15 leads to retinal dysfunction in Pcdh15R250X mutant mice.

Figure supplement 4- source data 1. Excel sheet showing data from experiments plotted in Figure 2-figure supplement 4b, c, d.
Figure 3: Loss of protocadherin-15 leads to aberrant localization of key proteins involved in the phototransduction cascade and retinoid cycle. a, Representative confocal micrographs of light adapted retinas shows mislocalization of phototransduction cascade proteins, arrestin and transducin, to both the inner segment (IS) and outer segment (OS) in mutant mice (right panels). In control mice, transducin is correctly localized to the IS and arrestin is to the OS (left panels). A schematic of the localization of arrestin and transducin in control and mutant mice is also shown. Scale bar – 20 µm. ONL – outer nuclear layer, OPL – outer plexiform layer. b, c Immunoblot of proteins involved in the visual retinoid cycle shows reduced quantities of RPE65 and CRALBP but not IRBP, quantified in c. d Quantification of indicated retinoid species from control and mutant mice shows reduced quantities of 11-cis retinal oxime. Data presented as mean ± SEM. Each data point represents an individual mouse. Student unpaired t-test, \( p < 0.05 \) (*) \( < 0.01 \) (**).

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Source data 1. Excel sheet showing data from experiments plotted in Figure 3b, c, d.

Figure supplement 1. Loss of protocadherin-15 does not affect dark adapted localization of key phototransduction cascade proteins.

Figure supplement 1-source data 1. Excel sheet showing data from experiments plotted in Figure 3-figure supplement 1c, d, e.

Figure supplement 2. Loss of protocadherin-15 leads to no apparent RPE degeneration in \( Pcdh15^{R250X} \) mutant mice.
**Figure 4: Exogenous 9-cis retinal rescues ERG deficits in young and old mutant mice**

a, d Representative scotopic ERG traces from young (2-3 months) a and old (6-7 months) d 9-cis retinal injected Pcdh15 mutant mice (right panels) show waveforms comparable to vehicle injected control mice (left panels). Same Pcdh15R250X mutant mice assessed 1 week prior to 9-cis retinal injection and ERG assessment show significantly reduced waveforms (central panels, baseline). b, e a- (left panel) and b-wave (right panel) quantification of scotopic ERG amplitudes shown in a and d, respectively. c, f Quantification of photopic b-wave for the denoted mice shows 9-cis retinal also improved cone-mediated function of mutant mice. f a- (left panel) and b-wave (right panel) quantification of scotopic ERG amplitudes for a different cohort of 6-7-months-old mice shows that 2 weeks after 9-cis retinal injection in mutant mice, the efficacy start to wane. Data presented as mean ± SEM. One-way ANOVA and Bonferroni post hoc test, $p<0.05$ (*) or $<0.001$ (**). NS – not significant.

The online version of this article includes the following source data and figure supplement(s) for figure 4:

**Source data 1.** Excel sheet showing data from experiments plotted in Figure 4b, c, e, f, g.

**Figure supplement 1.** Accumulation of various retinoids in tissues following exogenous 9-cis retinal delivery.

**Figure supplement 1-source data 1.** Excel sheet showing data from experiments plotted in Figure 4-figure supplement 1a, b.

**Figure supplement 2.** Exogenous 9-cis retinal does not improve the mislocalization of key phototransduction cascade proteins.

**Figure supplement 2-source data 1.** Excel sheet showing data from experiments plotted in Figure 4-figure supplement 2b.
**Figure 1**-**Figure supplement 1**: Fundus autofluorescence, Optical Coherence Tomography, and electroretinography in compound heterozygous patient LMG197#1831.

a-c: Left eye fundus autofluorescence in C. shows a central area, delineated by white arrowheads, of complete loss of autofluorescence consistent with RPE atrophy (compare with normal fundus autofluorescence in a.). The images were obtained at the most recent patient visit (age 50 yrs). d. Left eye Optical Coherence Tomography, also obtained at 50 yrs of age, shows the corresponding macular area with complete RPE and outer retinal atrophy as indicated by homogenous choroidal hypertransmission (white arrowheads) and absence of RPE band (in addition to significant macular thinning and loss of photoreceptors). Contrast with normal OCT anatomy presented in panel b. e. Electroretinography from the same patient at 38 yrs of age with noise level (extinguished) responses in both eyes. Median and 95% confidence interval for amplitude and implicit time indicated by the gray bars and orange line for the median. An example of normal waveform tracing is also shown.

**Figure 2** - **Figure supplement 1**: Validation of loss of protocadherin-15 in the retina and cochlea of Pcdh15R250X mutant mice

a Confocal micrographs of P97 mouse retinas from control and Pcdh15R250X mutants immunostained for protocadherin-15 (PCDH15, cyan, left panel) and opsin to label outer segments (OS, magenta, center panel) shows robust protocadherin 15 localized to the inner segments (IS), outer plexiform layer (OPL) and ganglion cell layer (GCL). Notably, protocadherin-15 immunoreactivity is absent in Pcdh15R250X mutant mice. INL – inner nuclear layer; IPL – inner plexiform layer. b Zoomed in image of the OS/ IS interface. DAPI to visualize nuclei in grey. c Confocal micrographs of P60 organ of Corti of indicated genotype
immunostained with protocadherin-15 (PCDH15) antibody HL5614 (green) and counterstained with phalloidin (red) confirms the loss of protocadherin-15 in Pcdh15^{R250X/R250X} mice. Scale bar, 10 μm.

**Figure 2 - Figure supplement 2:** Pcdh15^{R250X} mutant mice have profound hearing loss and severe vestibular system dysfunction

**a** Average thresholds of acoustic brainstem response (ABR) to broadband clicks and tone-pips with frequencies of 8 kHz, 16 kHz, and 32 kHz in control (Pcdh15^{R250X/+}; black) and mutant (Pcdh15^{R250X/R250X}; red) mice at P16-P30 (n = 4/ genotype), revealed no detectable hearing in Pcdh15^{R250X} mutants. **b** Representative open-field exploratory behavior of a 2-month old mouse for denoted genotypes shows increased circling behavior in mutant mice (left panels), quantified in right panel. (n = 3/ genotype). **c** Average score of tail-suspension test in control and mutants at P16-30, further confirm vestibular areflexia in Pcdh15^{R250X} mutant mice. (n = 4/ genotype). Data are shown as Mean ± SEM. (***, p<0.001) using Student unpaired t-test. **d** Epifluorescence micrographs and corresponding DIC micrographs of control and Pcdh15^{R250X/R250X} cultured organ of Corti and vestibular explants imaged after exposure to 3μM of FM1-43, a channel permeable dye, for 10 sec, revealed impaired mechanotransduction function in Pcdh15^{R250X} mutant mice. The samples were dissected at P0 and kept two days in vitro (P0+ 2DIV). Scale bar: 20 μm.

**Figure 2 - Figure supplement 3:** Pcdh15^{R250X} mutant mice have degeneration of sensory hair cells in the organ of Corti **a**, **b** Confocal micrographs of P60 organ of Corti **a** and vestibular end organs **b** of control (Pcdh15^{R250X/+}) and mutant (Pcdh15^{R250X/R250X}) mice immunostained with myosin VIIa antibody (green) and counterstained with phalloidin (red) and
DAPI (blue), revealed degeneration of sensory hair cells. Medial and basal turns of the cochlea and utricle are shown. Scale bar, 10 μm.

Figure 2 - Figure supplement 4: Loss of protocadherin-15 leads to retinal dysfunction in Pcdh15^{R250X} mutant mice

a Representative ERG waveforms from 1-month old mice of the denoted genotypes show normal wave architecture with significantly reduced waveforms for mutant mice. b b/a ratio shows the mutant mice have a ratio comparable to control mice. c Recovery of scotopic ERG maximal a-wave amplitudes (a-wave ratio; mean ± SEM) after strong bleaching (estimated >90%) of rhodopsin in overnight dark-adapted control (Pcdh15^{R250X/+}) and mutant (Pcdh15^{R250X/R250X}) mice. Bleaching was achieved by a 45-s illumination with bright white light at time 0. d Quantification of single flash pre-bleach a-wave amplitudes from the same mice as shown in c. Although the recovery of a-wave amplitude (ratio of pre-bleach to post-bleach) in both control and mutant was similar, the initial single flash a-waves were significantly reduced in mutant mice.

Figure 3 - Figure supplement 1: Loss of protocadherin-15 does not affect dark adapted localization of key phototransduction cascade proteins

a Representative confocal micrographs of dark adapted retinas show the correct localization of phototransduction cascade proteins, transducin to the outer segment (OS) and arrestin to the inner segment (IS), in both control and mutant mice. Schematic of the localization of arrestin and transducin in control and mutant mice is also shown. b Dark adapted (top panels) and light adapted (bottom panels) localization of opsin is not affected by loss of protocadherin-15.
Schematic of the localization of opsin in control and mutant mice is also shown. Scale bar – 20 µm. ONL – outer nuclear layer, OPL – outer plexiform layer. c-e Quantification of denoted proteins in the inner segment (IS) or outer segment (OS) for panels in a, b and Fig. 3a shows mislocalization of transducin to the OS and arrestin to the IS in mutant mice. Student unpaired t-test, $p < 0.05$ (*), $p < 0.001$ (***) NS – not significant.

Figure 3 - Figure supplement 2: Loss of protocadherin-15 did not apparent degeneration of RPE in $Pcdh15^{R250X/R250X}$ mutant mice. Transmission electron micrographs of 1-2 months old mice of denoted genotype shows normal RPE with no obvious structural abnormalities, irrespective of genotype. Scale bar – 2 µm. Data presented as mean ± SEM. Each data point represents an individual mouse. Student unpaired t-test, $p<0.01$ (**).

Figure 4 - Figure supplement 1: Accumulation of various retinoids in tissues following exogenous 9-cis retinal delivery. a, eye; and b, liver. Student unpaired t-test, $p < 0.05$ (*), ns – not significant.

Figure 4 - Figure supplement 2: Exogenous 9-cis retinal does not improve the mislocalization of key phototransduction cascade proteins a Representative confocal micrographs of light adapted retinae from vehicle injected control mice ($Pcdh15^{R250X/+}$), exhibit the correct localization of phototransduction cascade proteins, transducin to the outer segment (OS) and arrestin to the inner segment (IS). 9-cis retinal injected mutant mice ($Pcdh15^{R250X/R250X}$) exhibit the same aberrant mislocalization of arrestin and transducin. Schematic of the localization of arrestin and transducin in control and mutant mice is
also shown. Scale bar – 10 µm. 

Quantification of denoted proteins in the IS or the OS for panels in a. Student unpaired *t*-test, ns – not significant.
a

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Figure a: Graph showing the a-wave recovery after 90% bleach for different genotypes.

Figure b: Graph showing the b/a-wave ratio across different light intensities.

Figure c: Graph showing the a-wave amplitude over time after bleach for different genotypes.

Figure d: Graph showing the single flash pre-bleach a-wave amplitude for different genotypes.
A

\[ \text{Pcdh}15^{R250X+/+} \]: vehicle injected, n=5

\[ \text{Pcdh}15^{R250X/R250X} \]: 9-cis retinal injected, n=5

- 9-cis retinal: ns
- 11-cis retinal: ns
- all-trans retinal: *
- retinyl esters: ns

B

- all-trans retinol: ns
- retinyl esters: *
- 9-cis retinal: *
a

Light adapted

Pcdh15\textsuperscript{R250X/R250X} vehicle injected

\begin{tabular}{ccc}
  & OS & IS \\
OPL & & IS \\
ONL & & OS \\
\end{tabular}

Pcdh15\textsuperscript{R250X/R250X} 9-cis retinal injected

\begin{tabular}{ccc}
  & OS & IS \\
OPL & & IS \\
ONL & & OS \\
\end{tabular}

b

\begin{figure}
  \centering
  \includegraphics[width=\textwidth]{chart.png}
  \caption{Graph showing arbitrary fluorescence units for different conditions.}
  \label{fig:chart}
\end{figure}

\begin{itemize}
  \item Pcdh15\textsuperscript{R250X/R250X}; vehicle injected; n=4
  \item Pcdh15\textsuperscript{R250X/R250X}; 9-cis retinal injected; n=4
\end{itemize}