Supplementary Data

Supplementary Methods

Genetic analyses

Whole venous blood samples were collected and genomic DNA was extracted according to standard procedures. The structure and integrity of the *OPN1LW/OPN1MW* gene cluster on the X-chromosome were investigated by three methods, representing different groups of authors: Method 1 (performed by S.K. and B.W. in 12 of the 20 patients; Patients 1–4, 7–10, 15, 16, 19, 20) used a two-tiered testing scheme. The first-tier screened for large genomic deletions at the *OPN1LW/OPN1MW* gene cluster and the upstream locus control region (LCR), the presence of single or multiple opsin gene copies, and the presence or absence of the common point mutation c.607T>C p.C203R. Briefly, duplex polymerase chain reactions (PCRs) including primers for the tested fragment and an autosomal control fragment were performed for (1) a sequence defined as the common overlap of known deletions of the LCR (Nathans *et al.*, 1989, 1993), (2) the *OPN1LW* promoter, and (3) the *OPN1MW* promoter to test for large genomic deletions and the presence of single or multiple opsin gene copies. In addition, two PCR fragments for exons 4 and 5, respectively, for both *OPN1LW* and *OPN1MW* were amplified and digested with *Bst*UI and *Rsa*I, respectively. The restriction fragment length polymorphism (RFLP) in exon 4 via *BstUI* analyzed for the c.607T>C p.C203R mutation, while the RFLP in exon 5 via *RsaI* addressed the dimorphism at amino acid position 277—p.Y277 being specific for the *OPN1LW* gene and p.F277 for the *OPN1MW* gene. Second-tier testing (not necessary for the cases reported in this study) analyzed for other deleterious point mutations or haplotypes in cases with either single or multiple structurally intact gene copies. Primers and conditions are available upon request.

In Method 2 (performed by R.B.H. and Z.M.A. in 7 of the 20 patients; Patients 5, 6, 11–13, 17, 18), there was sequencing of the LCR, promoter, and *OPN1MW* and *OPN1LW* genes. Primers flanking 300–500 bp segments were designed to amplify exons, the immediate 500 bp of 5′ promoter, the 37 bp core LCR sequence, and interval sequences spaced every ~1 kb from the promoter to ~9 kb upstream of the first opsin gene. PCR and DNA sequencing was performed as described (Jaworek *et al.*, 2012). Briefly, PCR products were amplified using 50 ng DNA, with standard PCR reagents (Genscript) or AmpliTaq gold (Invitrogen), on an ABI Veriti Thermocycler (Applied Biosystems). A portion of amplified products were electrophoresed and measured on a 2% agarose gel. PCR products were precipitated and sequencing PCR performed using BigDye Terminator Ready Reaction Mix (ABI Biosystems). PCR primer sequences and conditions are available upon request. To evaluate for deletions, the presence or absence of PCR products was checked—spaced every ~1 kb of the 9 kb region upstream of the first opsin gene. Deletion size was estimated by the absence of sequential PCR products (assumed to be encompassed by the deletion). To confirm the absence of the LCR, three overlapping primer pairs were designed to amplify the core 37 kb segment and surrounding LCR. Deletion endpoints were mapped by designing forward and reverse primers flanking the region of the absent PCR products. Real-time PCR (RT-PCR) was used to determine the number of genes in the array, and the order of *OPN1LW* and *OPN1MW* genes was determined (Neitz and Neitz, 2011). Briefly, a quantitative RT-PCR assay was employed to estimate the relative ratio of first versus downstream genes in the X-chromosome opsin gene arrays. Quantitative RT-PCR was also used to estimate the relative ratio of L to M genes in each X-chromosome array.

Method 3 (performed by J.C.G. and A.J.H. in P14) has been described (Gardner *et al.*, 2010).

In vivo *microscopy of human retina*

Cross-sectional images of the retina were obtained with a spectral-domain optical coherence tomography (OCT) instrument (RTVue-100; Optovue Inc.). Some ONL data were obtained with other OCT systems (Bioptigen Inc. and Heidelberg Engineering GmbH). Overlapping, nonaveraged, 9-mm-long scans were used to study the retina along the vertical meridian through the fovea. Raster scans (6×6 mm, 101 lines with 513 longitudinal reflectivity profiles [LRPs] each) centered at the fovea provided high-density coverage of the macula. Scans were analyzed with custom programs (MatLab 7.5; MathWorks). For the outer retinal sublaminae, signal peak assignments were based on our previously published work (Aleman *et al.*, 2008; Jacobson *et al.*, 2009, 2013; Maeda *et al.*, 2009; Cideciyan *et al.*, 2011; Sakami *et al.*, 2011) and those of others (Hood *et al.*, 2009; Zhang *et al.*, 2011; Birch *et al.*, 2013) and were consistent with hypotheses on the correspondence between OCT signals and histologically defined layers (Huang *et al.*, 1998; Jacobson *et al.*, 2003; Spaide and Curcio, 2011) and interpretations of ultrahigh resolution AO OCT scans (Kocaoglu *et al.*, 2011). Specifically, outer retinal sublaminae were assumed to include (a) a hyperreflective layer at the outer plexiform layer; (b) a hyporeflective layer at the outer nuclear layer; (c) a hyperreflective layer at the outer limiting membrane; (d) a hyperreflective layer near the junction of inner and outer segments possibly corresponding to the ellipsoid region of inner segments; (e) a hyperreflective layer near the interface between cone outer segments and contact cylinder (extrafoveal) or apical processes (foveal) of the retinal pigment epithelium (RPE); (f) a hyperreflective layer near the interface between rod outer segments and RPE apical processes; and (g) a hyperreflective layer near the interface between basal RPE and Bruch membrane. Layer (f) could merge into layer (e) and become a single layer at the foveola in normal subjects. Outer nuclear layer thickness was defined as the distance from (a) to (c), cone outer segment thickness from (d) to (e), and rod outer segment thickness from (d) to (f). Normal subjects ($n=22$; age range 8–62 years) included a subset ($n=15$) with spherical errors +2 to −4 D, and another subset ($n=7$) with high myopia, −5 to −10 D.

Adaptive optics imaging of the photoreceptor mosaic

A previously described adaptive optics scanning laser ophthalmoscope was used to image each subject's photoreceptor

mosaic (Dubra and Sulai, 2011). Image sequences of 150 frames were recorded at each retinal location using a 775 or 796 nm superluminescent diode, and an internal fixation target was used to guide the subjects' fixation for imaging of different retinal locations. Intraframe distortion caused by the sinusoidal motion of the resonant optical scanner was estimated from images of a Ronchi ruling and subsequently removed by resampling each frame of the raw video over a square pixel grid. After desinusoiding, a reference frame with minimal intraframe eye motion was manually selected from each image sequence for subsequent registration using a previously described strip-based registration method (Dubra and Harvey, 2010). Each frame was divided into strips, and each strip was registered against the reference frame by finding the relative position that maximized the normalized cross-correlation between them (Dubra and Harvey, 2010). The registered frames were then averaged to create a single high signal-to-noise image for each image sequence, with between 40 and 50 frames being averaged to produce the final image at each retinal location.

The registered images for each subject were combined into a single montage (Adobe Photoshop; Adobe Systems, Inc.), which was then registered to an image of the fundus exported from the Cirrus HD-OCT system (Carl Zeiss Meditec). This was done to determine the absolute location of the individual images within the montage with respect to the fovea. Axial length measurements were obtained for all subjects using an IOL Master (Carl Zeiss, Inc.). Normal subjects ($n = 17$; age range 20–36 years; axial length 22.46–27.98 mm) were imaged as controls: 11 were imaged at 0.5 and 0.8 mm, 4 were imaged at 1.5 mm, 1 was imaged at all three parafoveal locations, and 1 was imaged at the foveal center and 0.5 and 0.8 mm.

For 13 of the controls and 2 patients (P6, P11), cone density was measured at 0.5 and 0.8 mm superior to the fovea using manual selection of cones, and in five of the controls and one of the patients (P11), both cone and rod density was measured at 1.5 mm. In the parafovea, density was measured over an $80\ \mu m \times 80\ \mu m$ sampling window, which was chosen because the cone mosaic was relatively sparse in the two patients, and it was necessary to use a sampling window large enough to avoid capturing local inhomogeneity of the residual cone mosaic.

Spectral sensitivity function

Spectral sensitivity functions were measured in normal subjects ($n = 3$, age range 31–50) and BCM P15 using a modified perimeter with methods previously described (Cideciyan *et al.*, 1997, 1998, 2000; Jacobson *et al.*, 2013). In brief, a 1.7° (equivalent to ~ 0.5 mm on the retina) diameter, 200-msec-duration test stimulus spectrally shaped by interference filters (7–12 nm full width at half maximum, depending on wavelength) was used. In normal subjects, stimuli were presented at 0.6 mm superior retina with the use of an offset dim fixation. In BCM, stimuli were presented at the center of four dim fixation lights. Thresholds are reported normalized to energy. Spectral sensitivity functions for rods and S and L/M cones are also shown in terms of relative energy. Spectral sensitivity functions were recorded under dark-adapted conditions and on increasing levels of achromatic backgrounds. Sensitivities were fit by eye with scotopic and photopic luminosity functions, and S-cone spectral sensitivity function (Stockman *et al.*, 1999). Note that normal subtractive cone–cone interactions on brighter background lights cause relative reduction of the sensitivity to the 560 nm stimulus compared with neighboring 500 and 600 nm samples (Sperling and Harwerth, 1971).

Supplementary References

Aleman, T.S., Cideciyan, A.V., Sumaroka, A., *et al.* (2008). Retinal laminar architecture in human retinitis pigmentosa caused by rhodopsin gene mutations. Invest. Ophthalmol. Vis. Sci. 49, 1580–1590.

Birch, D.G., Locke, K.G., Wen, Y., *et al.* (2013). Spectral-Domain optical coherence tomography measures of outer segment layer progression in patients with X-Linked retinitis pigmentosa. JAMA Ophthalmol. 131, 1143–1150.

Cideciyan, A.V., Pugh, E.N., Jr., Lamb, T.D., *et al.* (1997). Rod plateaux during dark adaptation in Sorsby's fundus dystrophy and vitamin A deficiency. Invest. Ophthalmol. Vis. Sci. 38, 1786–1794.

Cideciyan, A.V., Zhao, X., Nielsen, L., *et al.* (1998). Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man. Proc. Natl. Acad. Sci. USA 95, 328–333.

Cideciyan, A.V., Haeseleer, F., Fariss, R.N., *et al.* (2000). Rod and cone visual cycle consequences of a null mutation in the 11-cis-retinol dehydrogenase gene in man. Vis. Neurosci. 17, 667–678.

Cideciyan, A.V., Rachel, R.A., Aleman, T.S., *et al.* (2011). Cone photoreceptors are the main targets for gene therapy of NPHP5 (IQCB1) or NPHP6 (CEP290) blindness: generation of an all-cone Nphp6 hypomorph mouse that mimics the human retinal ciliopathy. Hum. Mol. Genet. 20, 1411–1423.

Dubra, A., and Harvey, Z. (2010). Registration of 2D images from fast scanning ophthalmic instruments. The 4th International Workshop on Biomedical Image Registration, Lübeck, Germany.

Dubra, A., and Sulai, Y. (2011). Reflective afocal broadband adaptive optics scanning ophthalmoscope. Biomed. Opt. Express 2, 1757–1768.

Gardner, J.C., Webb, T.R., Kanuga, N., *et al.* (2010). X-linked cone dystrophy caused by mutation of the red and green cone opsins. Am. J. Hum. Genet. 87, 26–39.

Hood, D.C., Lin, C.E., Lazow, M.A., *et al.* (2009). Thickness of receptor and post-receptor retinal layers in patients with retinitis pigmentosa measured with frequency-domain optical coherence tomography. Invest. Ophthalmol. Vis. Sci. 50, 2328–2336.

Huang, Y., Cideciyan, A.V., Papastergiou, G.I., *et al.* (1998). Relation of optical coherence tomography to microanatomy in normal and rd chickens. Invest. Ophthalmol. Vis. Sci. 39, 2405–2416.

Jacobson, S.G., Cideciyan, A.V., Aleman, T.S., *et al.* (2003). Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. Hum. Mol. Genet. 12, 1073–1078.

Jacobson, S.G., Aleman, T.S., Sumaroka, A., *et al.* (2009). Disease boundaries in the retina of patients with Usher syndrome caused by MYO7A gene mutations. Invest. Ophthalmol. Vis. Sci. 50, 1886–1894.

Jacobson, S.G., Cideciyan, A.V., Peshenko, I.V., *et al.* (2013). Determining consequences of retinal membrane guanylyl cyclase (RetGC1) deficiency in human Leber congenital amaurosis en route to therapy: residual cone-photoreceptor vision

correlates with biochemical properties of the mutants. Hum. Mol. Genet. 22, 168–183.

Jaworek, T.J., Kausar, T., Bell, S.M., *et al.* (2012). Molecular genetic studies and delineation of the oculocutaneous albinism phenotype in the Pakistani population. Orphanet. J. Rare. Dis. 7, 44.

Kocaoglu, O.P., Lee, S., Jonnal, R.S., *et al.* (2011). Imaging cone photoreceptors in three dimensions and in time using ultrahigh resolution optical coherence tomography with adaptive optics. Biomed. Opt. Express 2, 748–763.

Maeda, T., Cideciyan, A.V., Maeda, A., *et al.* (2009). Loss of cone photoreceptors caused by chromophore depletion is partially prevented by the artificial chromophore pro-drug, 9-cis-retinyl acetate. Hum. Mol. Genet. 18, 2277–2287.

Nathans, J., Davenport, C.M., Maumenee, I.H., *et al.* (1989). Molecular genetics of human blue cone monochromacy. Science 245, 831–838.

Nathans, J., Maumenee, I.H., Zrenner, E., *et al.* (1993). Genetic heterogeneity among blue-cone monochromats. Am. J. Hum. Genet. 53, 987–1000.

Neitz, J., and Neitz, M. (2011). The genetics of normal and defective color vision. Vis. Res. 51, 633–651.

Sakami, S., Maeda, T., Bereta, G., *et al.* (2011). Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. J. Biol. Chem. 286, 10551–10567.

Spaide, R.F., and Curcio, C.A. (2011). Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: literature review and model. Retina 31, 1609–1619.

Sperling, H.G., and Harwerth, R.S. (1971). Red-green cone interactions in the increment-threshold spectral sensitivity of primates. Science 172, 180–184.

Stockman, A., Sharpe, L.T., and Fach, C. (1999). The spectral sensitivity of the human short-wavelength sensitive cones derived from thresholds and color matches. Vis. Res. 39, 2901–2927.

Zhang, X., Ramachandran, R., Talamini, C.L., *et al.* (2011). The inner segment/outer segment border seen on optical coherence tomography is less intense in patients with diminished cone function. Invest. Ophthalmol. Vis. Sci. 52, 9703–9709.