Retinal Laminar Architecture in Human Retinitis Pigmentosa Caused by *Rhodopsin* Gene Mutations

Tomas S. Aleman,¹ Artur V. Cideciyan,¹ Alexander Sumaroka,¹ Elizabeth A. M. Windsor,¹ Waldo Herrera,¹ D. Alan White,² Shalesh Kaushal,² Anjani Naidu,¹ Alejandro J. Roman,¹ Sharon B. Schwartz,¹ Edwin M. Stone,^{3,4} and Samuel G. Jacobson¹

PURPOSE. To determine the underlying retinal micropathology in subclasses of autosomal dominant retinitis pigmentosa (ADRP) caused by *rhodopsin* (*RHO*) mutations.

METHODS. Patients with *RHO*-ADRP ($n = 17$, ages 6–73 years), representing class A (R135W and P347L) and class B (P23H, T58R, and G106R) functional phenotypes, were studied with optical coherence tomography (OCT), and colocalized visual thresholds were determined by dark- and light-adapted chromatic perimetry. Autofluorescence imaging was performed with near-infrared light. Retinal histology in hT17M-*rhodopsin* mice was compared with the human results.

RESULTS. Class A patients had only cone-mediated vision. The outer nuclear layer (ONL) thinned with eccentricity and was not detectable within 3 to 4 mm of the fovea. Scotomatous extracentral retina showed loss of ONL, thickening of the inner retina, and demelanization of RPE. Class B patients had superior-inferior asymmetry in function and structure. The superior retina could have normal rod and cone vision, normal lamination (including ONL) and autofluorescence of the RPE melanin; laminopathy was found in the scotomas. With Fourier-domain-OCT, there was apparent inner nuclear layer (INL) thickening in regions with ONL thinning. Retinal regions without ONL had a thick hyporeflexive layer that was continuous with the INL from neighboring regions with normal lamination. Transgenic mice had many of the laminar abnormalities found in patients.

CONCLUSIONS. Retinal laminar abnormalities were present in both classes of *RHO*-ADRP and were related to the severity of colocalized vision loss. The results in human class B and the transgenic mice support the following disease sequence: ONL diminution with INL thickening; amalgamation of residual ONL with the thickened INL; and progressive retinal remodeling with eventual thinning. (*Invest Ophthalmol Vis Sci.* 2008;49:1580–1590) DOI:10.1167/iovs.07-1110

From the ¹Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania; the ²Department of Ophthalmology and The Charlie Mack Overstreet Laboratories for Retinal Diseases, University of Florida, Gainesville, Florida; and the ³Howard Hughes Medical Institute and ⁴Department of Ophthalmology, University of Iowa Carver College of Medicine, Iowa City, Iowa.

Supported by grants from the Foundation Fighting Blindness, Macula Vision Research Foundation, Hope for Vision, Macular Disease Foundation, Research to Prevent Blindness, Ruth and Milton Steinbach Fund, and Alcon Research Institute.

Submitted for publication August 23, 2007; revised November 8, 2007; accepted February 22, 2008.

Disclosure: T.S. Aleman, None; A.V. Cideciyan, None; A. Sumaroka, None; E.A.M. Windsor, None; W. Herrera, None; D.A. White, None; S. Kaushal, None; A. Naidu, None; A.J. Roman, None; S.B. Schwartz, None; E.M. Stone, None; S.G. Jacobson, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Tomas S. Aleman, Scheie Eye Institute, University of Pennsylvania, 51 N. 39th Street, Philadelphia, PA 19104; aleman@mail.med.upenn.edu.

Mutations in *rhodopsin* (*RHO*), the gene encoding the rod photoreceptor visual pigment, were the first molecular defects identified in retinitis pigmentosa (RP) and are the most frequent cause of autosomal dominant (AD) RP (reviewed in Refs. 1–4). Rhodopsin, a G-protein-coupled receptor (GPCR), has a long history of scientific investigation (summarized in Ref. 5). Insight into the role of mutant rhodopsin in retinal dysfunction and degeneration has been gained from studies in vitro^{6–9} and in animals with *rbo* mutations.^{10–19}

What do we know about human *RHO*-ADRP? More than 100 mutations in *RHO* cause ADRP^{1,3} (<http://www.sph.uth.tmc.edu/Retnet>; provided in the public domain by the University of Texas Health Science Center, Houston, TX) and the functional phenotype is not a single disease expression.^{1,20–26} Building on subclassification schemes of ungenotyped ADRP,^{27–30} we have proposed two main phenotypic classes of *RHO*-ADRP²⁴. Class A mutants lead to severely abnormal rod function across the retina from early life and the topography of residual cone function parallels cone density. Class B mutants can have nearly normal rods, even into adult life in some retinal regions or throughout the retina, and there is a slow stereotypical disease sequence with an intraretinal gradient of disease vulnerability.

The micropathology of human *RHO*-ADRP has not been fully characterized because of the limited availability and advanced disease stages of postmortem donor retinas.³¹ Certainly, the final common pathway of photoreceptor death in *RHO*-ADRP has been confirmed.^{31,32} Morphologic correlates of the intraretinal disease gradient in class B have been reported for T17M and P23H mutations.^{33,34} In the present work we used in vivo cross-sectional optical imaging to study the morphologic phenotype of *RHO*-ADRP at different disease stages and for different subclasses. With the ultimate goal of establishing criteria for feasibility of future treatment trials in these common retinal degenerations, we studied retinal laminar architecture in both classes of *RHO*-ADRP. Retinal histopathology in a murine model of class B *RHO*-ADRP¹⁹ was used to augment understanding of the human results.

METHODS

Human Subjects

There were 17 patients with *RHO*-ADRP (age range, 6–73 years), representing nine families (Table 1). Normal subjects ($n = 28$; age range, 5–58 years) were included. Informed consent (or assent) was obtained for all subjects; procedures adhered to the Declaration of Helsinki and were approved by the institutional review board.

Optical Coherence Tomography

Retinal cross-sections were obtained with optical coherence tomography (OCT). Most data were acquired with OCT3 (Carl Zeiss Meditec, Inc., Dublin, CA); in five patients, OCT1 was used. Our methods and analysis techniques have been published.^{35–38} A subset of patients ($n = 4$) and normal subjects ($n = 10$) had additional ultra-high-speed and higher-resolution OCT imaging with Fourier-domain (FD) OCT (RTVue-

TABLE 1. Clinical and Molecular Characteristics of the Patients

Family (Mutation), Patient	Age(y)/ Sex	Visual Acuity (RE, LE)*	Refraction†	Kinetic Visual Field Extent (V-4e)‡	ERG Amplitudes§	
					Rod b-Wave	Cone Flicker
Class A <i>RHO</i> -ADRP						
Family 1 (R135W)						
P1	14/F	20/40, 20/32	+1.50	28	NP	NP
P2	20/M	20/25	−8.25	57	ND	5
P3	46/F	20/40, 20/32	−0.75	4	ND	ND
Family 2 (R135W)						
P1	6/M	20/63,20/100	+1.00	53	ND	4
P2	40/F	20/100-LP	+1.00	4, ND	ND	ND
Family 3 (R135W)						
P1¶	52/F	20/50	+1.50	<1	ND	ND
Family 4 (P347L)						
P1	32/F	20/40, 20/32	−1.75	2	ND	ND
Family 5 (P347L)						
P1¶	62/F	LP	+0.25	ND	ND	ND
Family 6 (P347L)						
P1¶	54/F	20/80, HM	+2.00	<1	ND	ND
Class B <i>RHO</i> -ADRP						
Family 7 (P23H)						
P1	18/M	20/20	−4.25	84	NP	NP
P2	45/F	20/20	−6.50	21	ND	6
P3¶	73/F	LP	+3.25	ND	ND	ND
Family 8 (T58R)						
P1	35/M	20/125	−1.75	39	34	34
Family 9 (G106R)						
P1	38/F	20/20	−0.25	59	17	40
P2	42/F	20/20	Plano	93	37	82
P3	44/F	20/20	−0.75	93	55	81
P4	63/M	20/20	+3.75	79	25	38

ND, not detectable; NP, not performed; LP, light perception; HM, hand motions.

*Best corrected visual acuity.

†Spherical equivalent; average both eyes.

‡Expressed as a percentage of normal mean of V-4e target; 2 SD below normal equals 90%; average both eyes; similar in the two eyes unless specified.

§Expressed as a percentage of normal mean amplitude (rod = 292 μ V; cone flicker = 172 μ V); 2 SD below normal equals 67% for rod b-wave and 60% for cone flicker.

||Rod and cone ERGs recorded >10 years before OCTs.

¶Aphakic or pseudophakic.

100; Optovue Inc., Fremont, CA). The high-definition (HD) line protocol of the FD-OCT system was used to obtain 4.5-mm long scans composed of 4,091 A-scans acquired at 26,000 A-scans per second. Overlapping OCT scans of 4.5-mm length were used to cover the vertical meridian up to 9 mm eccentricity from the fovea.

Postacquisition processing of data was performed with custom programs (MatLab 6.5, MathWorks, Natick, MA). Longitudinal reflectivity profiles (LRPs) making up the OCT scans were aligned using a dynamic cross-correlation algorithm.³⁵ Lateral sampling density of the FD-OCT scans were reduced by averaging eight neighboring LRPs, to increase the signal-to-noise ratio. Quantitative measurements of retinal laminae were performed after further reduction of lateral sampling density (sampling bins = 0.15 mm for OCT1 and OCT3; 0.07 mm for FD-OCT) and by averaging repeated scans after lateral and axial alignment.³⁶ Overall retinal thickness was defined as the distance between the signal transition at the vitreoretinal interface (labeled T1, Ref. 35) and the major signal peak corresponding to the retinal pigment epithelium (RPE).³⁸ In normal subjects, the RPE peak was assumed to be the last peak within the two- or three-peak scattering signal complex (labeled outer retinal-choroidal complex [ORCC] in Ref. 35) deep in the retina. In patients, the presumed RPE peak was sometimes the only signal peak deep in the retina. If multiple major peaks were present, the RPE peak was specified manually by considering the properties of the backscattering signal originating from layers vitread and sclerad to it.

Two nuclear layers, the outer photoreceptor nuclear layer (ONL) and the inner nuclear layer (INL), were defined in regions of scans showing two parallel stereotypical hyporeflective layers sandwiched between the RPE and vitreoretinal interface.^{35–38} The boundaries of these two hyporeflective layers were defined by the minima and maxima of the signal slopes. Transition regions where there was change from two to one hyporeflective layer were present in patient scans. In all cases the single hyporeflective layer was laterally continuous with the INL. An inner retinal thickness parameter was defined as the distance between the signal transition at the vitreoretinal interface and the sclerad boundary of the INL or the single hyporeflective layer continuous with the INL.^{35,36,38}

Autofluorescence Imaging

Spatial topography of RPE health was estimated with a recently developed autofluorescence (AF) method^{39–41} using near-infrared (NIR) wavelength excitation, to avoid absorption of imaging light by rhodopsin and thus the possibility of accelerating the natural history of *RHO*-ADRP.^{18,24,42} NIR-AF images were obtained as 25-frame stacks at 4 frames/s for overlapping 30° × 30° regions of central retina. In each stack, those images without visible distortion were selected, spatially registered, and averaged. Averaged images of the neighboring regions were digitally mosaiced by manually specifying retinal landmark pairs. The resultant wide-field images were registered to OCT images using

landmarks (e.g., foveal depression and retinal blood vessels) visible in both modalities.

Psychophysics and Electroretinography

Patients underwent kinetic visual field testing, electroretinography (ERG), and dark- and light-adapted chromatic static threshold perimetry (500-, 600-, and 650-nm stimuli with 200-ms duration and 1.7° diameter). Thresholds were measured on a 12° grid across the field and at 2° intervals along the vertical and horizontal meridians in the same retinal regions as the OCT scans. In these profiles, long/middle wavelength (L/M) cone function was determined either with 650-nm stimuli in the dark-adapted state and compared with normal data determined during the cone plateau phase of dark adaptation after bleaching or with 600-nm stimuli in the light-adapted state. Techniques, data analyses, and normal results have been described.^{43–46}

Histology in Mice

Transgenic *mrbo*^{+/-} T17M mice (hT17M; age range, 4–6 months, *n* = 4)¹⁹ and wild-type (WT) control mice (age range, 4–6 months, *n* = 4) were used. Studies conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and had institutional approval. After the mice were killed, the eyes were enucleated and fixed as described.¹⁹ Vertical sections through the optic nerve were stained with hematoxylin and eosin. Contiguous fields (each 350 μ m in length) extending into the peripheral retina from each side of the optic nerve were imaged at 20 $\times$ magnification¹⁹ and digitally montaged to create a composite image of the entire vertical section.

RESULTS

Classes of Disease Expression in *RHO*-ADRP

The differences in functional phenotype between class A and class B *RHO*-ADRP²⁴ are illustrated in Figure 1. F(family)1, P(patient)1, representing class A, showed constricted kinetic fields (Fig. 1A) and no measurable rod function by dark-adapted perimetry (Figs. 1B, 1C). Cone sensitivity was near normal at fixation but declined rapidly with eccentricity (Figs. 1B, 1C). F7, P1, representing class B, had a superior-field absolute scotoma (Fig. 1A). Dark-adapted perimetry showed near normal rod sensitivity in the inferior field, but a steep decline superiorly. Cone sensitivity had a similar vertical gradient of loss (Figs. 1B, 1C).

Class A patients (*n* = 9) had a range of kinetic field extents, in keeping with the spectrum of ages and severities (Table 1). ERGs showed either no detectable waveforms to standard stimuli or abnormal cone ERGs as the only recordable waveform. Dark-adapted sensitivities were cone-mediated when measurable (data not shown). All class B (*n* = 8) patients (except F7,P3) had measurable kinetic fields, and there were abnormally reduced rod and cone ERGs in most patients. Two patients (F9,P2; F9,P3) had normal cone ERGs. Dark-adapted sensitivities were mainly rod- or mixed-mediated at extracentral loci with detectable function, and there was a wide range of rod sensitivity losses; cone sensitivity losses were less than 1 log unit (data not shown).

Distorted Retinal Laminae in Class A *RHO*-ADRP

Cross-sectional OCT images along the vertical meridian in representative class A patients were compared with those in a normal subject (Fig. 2A). Normal retina showed a foveal depression that was surrounded by thicker retina and then decreasing thickness with distance from the center. Laminar organization was evident with layers of low reflectivity (INL and ONL) and intervening layers of higher reflectivity (inner plexiform layer, IPL; outer plexiform layer, OPL). Near the retinal surface there was a broader band of high reflectivity

FIGURE 1. Classes of disease expression in *RHO*-ADRP exemplified by data from two patients. (A) Kinetic perimetry results from the right eye using two targets (V-4e and I-4e). (B) Static threshold perimetry results, dark-adapted (top) and light-adapted (bottom), are displayed as gray-scale maps of rod and cone sensitivity loss. The scale has 16 levels of gray, representing 0- to 30-dB losses (right). The physiological blind spot is represented as a black square at 12° in the temporal field. N, nasal; T, temporal; I, inferior; and S, superior visual field. (C) Dark-adapted two-color (500 and 650 nm) vertical sensitivity profiles in the patients (symbols) compared with lower limits of normal (thick gray lines) for rod-mediated sensitivity to the 500-nm stimulus and for cone-mediated sensitivity to the 650-nm stimulus at the cone plateau. The photoreceptor mediation at each locus, based on the sensitivity difference between the two colors is given: R, rod-mediated; M, mixed rod- and cone-mediated; and C, cone-mediated.

representing the nerve fiber layer (NFL). Deep in the retina, there is a multilayer complex that we termed the ORCC,³⁵ composed of signals originating from the outer limiting membrane, the photoreceptor inner-outer segment (IS-OS) interface, the RPE, and the anterior choroid.

The central retina of a class A patient (F2,P1, age 6) showed prominent cystoid changes (Fig. 2A). A thinned ONL was discernible (arrows). Cone sensitivity was reduced, and there was no measurable rod function. Eccentric to the cystoid changes, there was abnormal laminar architecture. The inner retinal hyper-reflectivity appeared thickened and the deeper low-reflectivity layer was unusually thick for INL. The patient's

40-year-old mother (F2,P2) also showed central cystoid changes. The ONL was barely discernible near the edge of the cystoid changes (Fig. 2A, arrow). Cone function was limited to a smaller central island than in her son. Extracentral, nonfunctioning retina also had abnormal-appearing laminae. A 54-year-old patient (F6,P1) showed a thinned fovea. The ONL was

barely detectable near the foveal center and was associated with a small area of cone function. At further eccentricities, the retina was thin and delaminated and deep backscatter was increased. Inferiorly, there were intraretinal hyper-reflectivities that may correspond to regions of pigment migration.

Quantitation of retinal thickness parameters in class A indicated that photoreceptor layer loss was accompanied by a thickened inner retina (Fig. 2B). The parameters were measured in the extracentral retina (>2 mm eccentricity), to avoid the distortion from cystoid changes, a frequent finding in class A (7/9 patients). Two older patients without cystoid changes showed abnormally thin foveas (F5,P1, 104 μm and F6,P1, 92 μm ; normal mean ± 2 SD = 207 ± 35 μm). Overall retinal thickness in all class A patients was either within normal limits or reduced. Many patients (6/9) had thin retinas at 2 to 3 mm eccentricity and progressively thicker retinas at more peripheral locations. ONL was not discernible (at the eccentricities quantified) in most (7/9) patients. In the youngest two patients (F1,P1; F2,P1), a thin ONL (<20 μm) extended to eccentricities between 2 and 4 mm (data not shown). The inner retina was abnormally thick at most locations in all patients (Fig. 2B).

Intraretinal Variation in Retinal Structure in Class B *RHO*-ADRP

Do the regional retinal differences determined by psychophysics in class B (Fig. 1) have structural correlates? Cross-sectional OCT images through the vertical meridian in class B patients representing early- and late-stage disease in the same family were compared with normal results (Fig. 3A). F7,P1 (age 18) showed a vertical gradient of structural change. There was normal lamination in a 6- to 7-mm region (Fig. 3A, white bracket) that extended from the fovea to ~ 4 mm into the superior retina, but only to ~ 2 mm inferiorly. The ONL was normal-appearing in this expanse of retina and there was near-normal rod and cone function. The superior retina beyond 4 mm showed retained lamination but gradual ONL thinning and loss of IS/OS signal. Inferior to 2 mm, there was an abrupt change in retinal structure with loss of normal lamination. This structural change was accompanied by diminished or nondetectable vision. The patient's grandmother (F7,P3, age 73) had vision limited to light perception. Her retina was thin and delaminated, with increased deep backscatter.

Quantitation of retinal thickness parameters in class B showed a vertical gradient of photoreceptor layer thinning and associated inner retinal abnormalities (Fig. 3B). The overall retinal thickness of F7,P1, the youngest patient studied, was normal or increased in thickness in paracentral locations, resembling early structural abnormalities in choroideremia.⁴⁷ In seven other patients with later-stage disease, retinal thickness

FIGURE 2. Retinal laminar architecture in class A *RHO*-ADRP. (A) Cross-sectional OCT images along the vertical meridian through the fovea in a normal subject (age 25; top) compared with three patients (bottom) representing different ages and disease stages in class A *RHO*-ADRP. Brackets defining ONL and the inner retina are labeled (left) and a bracket showing total retinal thickness is at the right edge. Bars above the scans show psychophysically determined rod (blue bar: dark-adapted, 500-nm stimulus) and cone (red bar: light-adapted, 600-nm stimulus) sensitivity. Arrows: discernible ONL in F2,P1 and F2,P2. (*) Cystoid changes. I, inferior; S, superior retina. Calibration bar at left. Inset: schematic location of the scans. (B) Thickness of the overall retina and inner retina along the vertical meridian at eccentricities >2 mm in 9 class A patients grouped by age. Measurements in some patients are interrupted in regions with or adjacent to cystoid changes. Shaded areas: normal limits (mean ± 2 SD) for retinal thickness ($n = 27$, ages 5–58) and inner retina ($n = 14$, ages 5–58). Insets: schematic location of the scans.

FIGURE 3. Retinal laminar architecture in class B *RHO*-ADRP. (A) Cross-sectional OCT images along the vertical meridian through the fovea in a normal subject (age 24; top) compared with two patients (bottom) representing class B patients. Brackets defining the ONL and the inner retina are labeled (left) and a bracket showing total retinal thickness is at the right edge. White bracket in the image obtained from F7,P1 delimits the segment with normal lamination. Bars above the scans indicate rod (blue) and cone (red) sensitivity (as in Fig. 2). I, inferior; S, superior. Left: calibration bar. Insets: schematic location of the scans. (B) Thickness of the overall retina, ONL, and inner retina along the vertical meridian in the eight patients. Shaded areas: normal limits (mean $\pm$ 2 SD) as in Fig. 2.

FIGURE 4. Topography of RPE disease in *RHO*-ADRP. (A–C) AF imaging results obtained with near-infrared (NIR) excitation in a 24-year-old normal subject (A) and in patients from the two classes of *RHO*-ADRP (B, C). The intensity of the NIR-AF image of F8,P1 (C) is shown scaled by 1.75 $\times$ compared with the normal subject (A) and F1,P1 (B) for better visualization of the regional features. (D) Map of mean rod photoreceptor density in the human retina (generated from data published in Ref. 48) for comparison with the NIR-AF images. Lighter intensities correspond to higher spatial densities ($\sim$ 130,000–160,000 rods/mm 2) and darker intensities to lower densities. Black oval: optic nerve head.

was normal or reduced, the latter occurring in older subjects. The ONL thickness within 1 to 2 mm of the fovea was normal in most (6/8) patients. In the superior retina the ONL thickness gradually declined with eccentricity. In the youngest subject the layer was measurable at 6 mm superior, but in others, the central island of ONL was separated from the more superior ONL by a region of undetectable ONL. At distances >5 mm from the fovea in the superior retina, the ONL and overall retinal thickness often were near normal. The inferior retinal ONL showed a steeper slope to thinning, being undetectable by $\sim$ 2 to 3 mm of eccentricity in all patients. The oldest subject (F7,P3) had ONL only at the fovea, and it was barely measurable. Inner retinal thickness was at or above the upper limit of normal across the region sampled in all patients. Hyperthick inner retina was commonly observed in younger patients. Of note, in superior regions with measurable ONL, the inner retina tended to be less thick than in other regions. The inferior inner retina at eccentricities beyond 3 to 4 mm eccentric was hyperthick in most (6/8) patients.

RPE Disease in *RHO*-ADRP and Relation to Photoreceptor Topography

NIR-AF was used to understand the spatial topography of RPE disease in *RHO*-ADRP (Fig. 4). In a normal subject (Fig. 4A), NIR-AF showed a central region of higher intensity surrounded by lower intensity extending peripherally. Normal NIR-AF signal is believed to be dominated by the AF of melanin and

melanolipofuscin in the RPE with a contribution from melanin in the choroid.^{39–41} Class A patient F1,P1 showed a central ellipsoid region of homogeneous appearing NIR-AF (Fig. 4B). Surrounding this region was a thin (~0.5 mm) transition band showing distinct hyperfluorescence. The paracentral retina peripheral to the transition band showed a spatially heterogeneous pattern of signal intensity that was probably dominated by the AF of choroidal melanin uncovered by the demelanization of the overlying RPE. Retinal and choroidal blood vessels appeared darker against the choroidal AF signal.⁴¹

Class B patient F8,P1 also showed a central region of relatively normal NIR-AF signal, suggesting RPE preservation (Fig. 4C). The preserved region was similar in size to that of the class A patient, but the shape was not as regular, and there was no apparent hyperautofluorescent transition band. At the fovea there were local losses of signal suggestive of focal foveal RPE disease, correlating with reduced visual acuity (Table 1) and a history of cystoid maculopathy. More peripherally, there was loss of NIR-AF signal, emergence of a blood vessel pattern similar to that in the class A patient, and thus delineation of regions of RPE demelanization. The choroidal AF signal was markedly lower in the superior retina than in the inferior retina, and that may suggest local choroidal atrophy. Further superiorly, at ~6 mm of eccentricity, the NIR-AF signal returned to the homogeneous appearance associated with normal RPE melanin. The inferior retina did not show this return to homogeneity (up to ~9.5 mm eccentricity that was imaged). The abnormal NIR-AF signal in the class B patient had distinct similarities in shape and location to those of the superior segment of the ring of high rod photoreceptor density in the normal retina⁴⁸ (Fig. 4D). Of interest, the superior region with the most abnormal NIR-AF signal in the class B patient corresponded to the normal “rod hotspot,”⁴⁸ a small region of highest rod density (Fig. 4D).

Further Dissecting the Laminopathy in *RHO*-ADRP with FD-OCT

Cross-sectional FD-OCT images along the vertical meridian in F1,P1 (class A) and F8,P1 (class B) were studied as inferior (Fig. 5) and superior (Fig. 6) sections. Normal inferior retina was clearly laminated (Fig. 5A). The class A patient showed central cystoid changes, but the ONL and INL were identifiable from ~2 to nearly 5 mm inferior to the fovea. There was no rod function, and cone function tapered to no perception at 4 to 5 mm. Further inferiorly, the “blind” retina was not normally laminated. There was mainly a hyper-reflective zone that was thicker than expected for the NFL and a thick hyporeflective layer that abutted the RPE and appeared continuous with the INL from more central retina. The class B patient had an abnormal central structure (history of cystoid maculopathy). A thin ONL and thickened INL were visible until ~3 to 4 mm inferior to the fovea; colocalized vision was only cone mediated. Further inferiorly, there were abnormalities comparable to those of the class A patient.

Quantitation of the images revealed that retinal thickness alone did not discriminate abnormalities (Fig. 5B). Eccentric to the central changes, the retina was normal or slightly thicker or thinner than normal. The ONL was reduced in both patients and could not be identified after ~3 to 4 mm. The INL was not measured in the region of cystoid change in the class A patient and was thicker than normal centrally in the class B patient. The region with undetectable ONL in both patients was associated with remarkably increased thickness of the retinal layer continuous with the INL.

The normal superior retina was clearly laminated (Fig. 6A). The class A patient showed laminar abnormalities in the superior retina similar to those found inferiorly. Eccentric to central

cystoid changes, the retinal layers were identifiable to 4 to 5 mm superior to the fovea. Further superiorly, there was “blind” and abnormally laminated retina. The class B patient also showed laminar architecture superior to the fovea (Fig. 6A) comparable to that inferior to the fovea (Fig. 5A). Abnormal lamination and diminished function occurred between the fovea and ~2 to 3 mm superiorly, and there was a zone of laminopathy from 3 to 5 mm. Eccentric to 5 mm, however, the ONL was again discernible, and the INL was thinner. These structural changes coincided with increased rod and cone vision.

Quantitation of the images (Fig. 6B) revealed that overall retinal thickness was deceptively normal beyond the central changes. The ONL was reduced in both patients and was undetectable after ~3 mm superior. In the class B patient, unlike the class A patient, the ONL became detectable again at 5 to 6 mm superiorly. The INL in the class B patient was measurable centrally and was thickened. In both patients, the area with undetectable ONL showed increased thickness of the retinal layer continuous with the INL. A remarkable difference in inner layer thickness was evident between the two patients at 5 to 8 mm superior. In the class B patient, coincident with the increased function and detectable ONL, there was distinguishable INL that was only modestly increased in thickness. In the class A patient, the inner hyporeflective layer was remarkably thick. In summary, FD-OCT demonstrated thickening of the INL and layers vitread to it in regions of ONL thinning or loss.

Laminopathy in the hT17M *Rho* Transgenic Mouse Retina

Do the laminar abnormalities in *RHO*-ADRP have any histologic correlates in a murine model in which a human rhodopsin transgene (hT17M) is expressed in a line of mice hemizygous null for wild-type mouse *rhodopsin* (*mrbo*^{+/-})?¹⁹ Histologic sections along the vertical meridian crossing through the optic nerve in a 6-month-old hT17M retina showed reduced ONL and photoreceptor IS/OS compared with WT retina (Fig. 7A). Magnified sections from a region ~400 to 500 μ m from the optic nerve in mutant mice illustrated that there was a range of ONL and IS/OS losses and accompanying inner retinal changes (Fig. 7B). A 4-month-old hT17M mouse had shortened IS/OS and reduced ONL thickness to approximately half that of the 4-month-old WT. The INL and IPL, however, were thicker than in the WT. Retinal sections from two 6-month-old hT17M mice illustrate the spectrum of structural changes observed at this age (Fig. 7B). The ONL and OPL were slightly thinner in one of these animals (Fig. 7Ba) compared with the younger counterpart, whereas the inner retina resembled that of WT. Sections from another 6-month-old hT17M mouse (Fig. 7Bb1, 7Bb2) at slightly different locations showed greater reduction of ONL, photoreceptor IS/OS length, and OPL. The INL appeared thicker than in WT (Fig. 7Bb1), and there was increased space between the INL nuclei. The latter feature was also apparent in the remainder of the sections from 6-month-old animals. A region with greater degeneration in the same animal showed groups of remaining photoreceptor nuclei (Fig. 7Bb2, arrows) in the ONL in proximity to the INL, with a thinned OPL and blurred boundary between these layers.

A sequence of structural changes from class B patient FD-OCT images is now postulated (Fig. 7C). The least affected region, 7-mm superior retina (Fig. 7C2), shows ONL loss to approximately one third of normal and no distinct IS/OS signal. The INL is thickened, as is the tissue vitread to it. This region resembles the section from the 4-month-old T17M mouse (Fig. 7B). A more advanced degenerative state is noted in a region 6 mm temporally (Fig. 7C3). Thickened INL is separated from the RPE by hyporeflective dots (Fig. 7C3, arrows) which may be

FIGURE 5. Detailed retinal structure of the inferior retina in *RHO*-ADRP examined by FD-OCT. (A) Cross-sectional FD-OCT along the vertical meridian from the fovea extending into the inferior retina in a normal subject (top) and two patients representing each class of *RHO*-ADRP (middle, bottom). Bars above the cross-sections indicate rod (blue) and cone (red) sensitivity (as in Fig. 2). Nuclear layers are labeled and highlighted (ONL, blue; INL and hyporeflexive layer continuous with it: purple). *Inset*: schematic location of the scans. Epiretinal membranes were visible in both patients. (★) Cystoid changes. *Left*: calibration bar. (B) Overall retinal, ONL, and INL thicknesses along the vertical meridian in the inferior retina in both patients. *Circles*: retinal regions with two detectable nuclear layers; *diamonds*: regions with a single hyporeflexive layer that is continuous with the INL from the more central retina. *Shaded areas*: normal limits (mean $\pm$ 2SD; n = 9, age range, 15–63).

remnant photoreceptor clumps, such as is found in mutant mouse retina (Fig. 7Bb2). The expanded INL is reminiscent of changes observed in both 6-month-old mutant mice (Figs. 7Bb1, 7Bb2). At a slightly more peripheral locus (Fig. 7C4), there is thinner retina with a bilaminar appearance. The deep hyporeflexivity may represent only INL or a combined INL and remnant ONL.

DISCUSSION

Class A *RHO*-ADRP, the phenotype with lack of rod function from early life,^{24,26} was accompanied by major losses of ONL.

Photoreceptor cell death is thus the basis of the well-described rod-mediated dysfunction, documented even in the first decade of life. Any therapy intended to preserve or restore rod photoreceptor function in this group of patients with *RHO*-ADRP would thus be impractical, unless data about the very early natural history of function and structure prove otherwise. Class A is further complicated by retinal structural abnormalities that likely represent neuronal–glial remodeling. This laminopathy has also been observed recently in two other forms of RP: recessive RP due to a *PDE6B* null mutation⁴⁹ and X-linked RP caused by *RPGR* mutations.³⁸ Other human retinal degenerations with documented laminopathy include Leber congenital

FIGURE 6. Detailed laminar structure of the superior retina in *RHO*-ADRP examined by FD-OCT. **(A)** Cross-sectional FD-OCT along the vertical meridian from the fovea extending into the superior retina in a normal subject (*top*) and two patients (*middle, bottom*; same subjects as Fig. 5) representing each class of *RHO*-ADRP. Bars above the cross-sections indicate rod (*blue*) and cone (*red*) sensitivity (as in Fig. 2). Nuclear layers are highlighted (ONL, *blue*; INL and hyporeflective layer continuous with it: *purple*). *Inset*: schematic location of the scans. Epiretinal membranes are visible in both patients. (*) Cystoid changes. *Left*: calibration bar. **(B)** Overall retinal thickness, ONL, and INL thicknesses along the vertical superior meridian in both patients. Symbols and shaded areas are as defined in Figure 5.

amaurosis^{36,50,51} and choroideremia.⁴⁷ Major rod receptor losses and retinal remodeling in class A *RHO*-ADRP, however, do not preclude all notions of therapy. Cone-specific therapy would be an appropriate direction in such patients. The extent of treatable retina should be measured so that expectations are realistic, and appropriate outcome measures are used to assay therapeutic effect.

Class B *RHO*-ADRP, the phenotype with major intraretinal differences in function ranging from near normal vision to absolute scotomas, showed micropathological features that warrant discussion. An annulus (~ 10 to 20° or 3–6 mm) of RPE abnormalities was observed with AF imaging in the region where normal retina shows highest rod densities.⁴⁸ This sug-

gests that RPE cell loss or depigmentation is occurring secondary to major rod photoreceptor loss. Although midperipheral scotomas ($\sim 30^\circ$ – 60° or 10–20 mm) are the traditional hallmark of RP,⁵² it is intriguing that we found evidence of severe disease more centrally in the class B *RHO*-ADRP patient. Maps of rod (and cone) sensitivity losses reported for class B patients at early disease stages have shown scotomas at test loci ~ 4 mm from fixation, encircling the fovea (Ref. 24, Figs. 2A, 3E; Ref. 26, Fig. 5B). The scotomatous retina in this region had little or no measurable ONL, and there was INL thickening. Evidence from these different functional and retinal-RPE structural modalities suggests a high vulnerability of the normally rod-dense annulus⁴⁸ in class B, with possibly a different natural history of

FIGURE 7. Comparison of histopathology of hT17M *rho* mutant mouse with results in a class B *RHO*-ADRP patient. (A) Low-magnification views of vertical retinal sections crossing the optic nerve from a 6-month-old hT17M *rho* transgenic mouse compared with an age-matched wild-type (WT) mouse. Yellow bracket: region examined at higher magnification in (B). (B) Magnified (40-μm-wide) retinal images taken at ~400 to 500 μm from the optic nerve in a 4-month-old mouse (Ba) and in two 6-month-old (Bb1, Bb2) transgenic mice compared to a 4-month-old WT mouse. (Bb2, arrows) Clusters of remaining nuclei in the ONL. (C) Cross-sectional, 500-μm-long FD-OCT images obtained at 5 to 9 mm of eccentricity in the superior and temporal retina in a class B *RHO*-RP patient compared with a normal subject. Schematic to the left depicts retinal regions sampled in each cross section. Reflectivity profiles (white traces) are overlaid on the FD-OCT scans; signal features representing nuclear layers are shown adjacent to highlighted layers (ONL, blue; INL and hyporeflective layer continuous with it: purple). Bars above the images indicate rod (blue) and cone (red) (as in Fig. 2) sensitivity. (C3, arrows) Hyporeflectivities that may correspond to clumps of remnant photoreceptor nuclei.

degeneration in regions with lesser rod density within and beyond this ring.

Eccentric to the rod ring defect, class B patients showed inferior-superior asymmetry of retinal structure and function. The inferior retina had demelanized RPE by AF imaging and abnormal laminar architecture by OCT. The primary rod disease in this region has been postulated to be exacerbated by stress from environmental light.^{18,20,21,23,24,33,42} The superior

retina could have normal RPE appearance and retinal structure. The transition from better to worse retinal-RPE health that was evident from superior retina (at ~6 mm eccentricity) toward more central retina is reminiscent of the border of light-accelerated lesions demonstrated histologically and with noninvasive imaging in the canine *rho* mutant model of Class B.¹⁸ Treatment strategies aimed at mutant rods would be best targeted to and evaluated at superior retinal regions with normal lamination, healthy RPE, demonstrable ONL, and rod-mediated function.

The present study is the second to seek understanding of OCT results in human retinal degeneration by comparison with histopathology in an animal model of the disease. We recently compared OCT abnormalities caused by mutations in *CEP290* with the *rd16* murine model. Evidence of remodeling like that predicted in humans was found in *rd16*.⁵³ The results from the transition zones in class B superior retina taken together with histopathology in the hT17M *rho* mutant mouse suggest a proposed sequence of retinal abnormalities that are detectable by OCT in human *RHO*-ADRP. Rod photoreceptor loss leads to diminished OPL and blurring of detectable boundaries between the thinned ONL and the thickened INL. The appearance becomes that of a single nuclear layer. Preservation or modest loss of cells within the inner retina has been reported in patients.^{23,31,33,54} The paradoxical increase in thickness of the INL observed may be related to Müller glial activation with hypertrophy, despite loss of cells therein.^{31,54} Although not the focus of other studies, INL/inner retinal thickening is evident in many histologic sections illustrating retinal degeneration in different animal models (e.g., Ref. 55, Fig. 2; Ref. 56, Fig. 2; Ref. 57, Fig. 1; Ref. 58, Fig. 1; Ref. 59, Fig. 7). Vitread to the apparently single nuclear layer is an abnormally thick hyperreflective layer with uncertain morphologic basis. One contributor to this increased thickness may be epiretinal membrane, a common finding in RP.³¹ Detection of the ganglion cell layer within this thickened tissue is a relevant future translational issue for treatment with visual prosthetic devices^{60,61} or strategies to convert ganglion cells or other inner retinal neurons to photosensitive cells by delivery of microbial-type rhodopsin by means of viral vectors.⁶² Late-stage retinal thinning, as we documented in both classes of *RHO*-ADRP, tends to be associated with less clear lamination and may be the *in vivo* microscopy version of remnant and migrated RPE with neural cells, neurite sprouting, and glial cells interspersed.³¹

Acknowledgments

The authors thank Elaine Smilko, Mary Nguyen, Michelle Doobrajh, and Malgorzata Swider for critical help.

References

- Gal A, Apfelstedt-Sylla E, Janecke AR, Zrenner E. Rhodopsin mutations in inherited retinal dystrophies and dysfunctions. *Prog Retinal Eye Res.* 1997;16(1):51–79.
- Kennan A, Aherne A, Humphries P. Light in retinitis pigmentosa. *Trends Genet.* 2005;21(2):103–110.
- Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet.* 2006;368(9549):1795–1809.
- Daiger SP, Bowne SJ, Sullivan LS. Perspective on genes and mutations causing retinitis pigmentosa. *Arch Ophthalmol.* 2007;125(2):151–158.
- Palczewski K, Hofmann KP, Baehr W. Rhodopsin—advances and perspectives. *Vision Res.* 2006;46(27):4425–4426.
- Sung CH, Davenport CM, Hennessey JC, et al. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proc Natl Acad Sci USA.* 1991;88(15):6481–6485.
- Sung CH, Davenport CM, Nathans J. Rhodopsin mutations responsible for autosomal dominant retinitis pigmentosa: clustering of

- functional classes along the polypeptide chain. *J Biol Chem*. 1993; 268(35):26645-26649.
8. Kaushal S, Khorana HG. Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa. *Biochemistry*. 1994;33(20):6121-6128.
 9. Deretic D. A role for rhodopsin in a signal transduction cascade that regulates membrane trafficking and photoreceptor polarity. *Vision Res*. 2006;46(27):4427-4433.
 10. Olsson JE, Gordon JW, Pawlyk BS, et al. Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. *Neuron*. 1992;9(5):815-830.
 11. Naash MI, Hollyfield JG, al-Ubaidi MR, Baehr W. Simulation of human autosomal dominant retinitis pigmentosa in transgenic mice expressing a mutated murine opsin gene. *Proc Natl Acad Sci USA*. 1993;90(12):5499-5503.
 12. Li, T, Snyder, WK, Olsson, JE, Dryja, TP. Transgenic mice carrying the dominant rhodopsin mutation P347S: evidence for defective vectorial transport of rhodopsin to the outer segments. *Proc Natl Acad Sci USA*. 1996;93(24):14176-14181.
 13. Humphries MM, Rancourt D, Farrar GJ, et al. Retinopathy induced in mice by targeted disruption of the rhodopsin gene. *Nat Genet*. 1997;15(2):216-219.
 14. Lewin AS, Drenser KA, Hauswirth WW, et al. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. *Nat Med*. 1998;4(8):967-971.
 15. Banin E, Cideciyan AV, Aleman TS, et al. Retinal rod photoreceptor-specific gene mutation perturbs cone pathway development. *Neuron*. 1999;23(3):549-557.
 16. Aleman TS, LaVail MM, Montemayor R, et al. Augmented rod bipolar cell function in partial receptor loss: an ERG study in P23H rhodopsin transgenic and aging normal rats. *Vision Res*. 2001; 41(21):2779-2797.
 17. Kijas JW, Cideciyan AV, Aleman TS, et al. Naturally occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa. *Proc Natl Acad Sci USA*. 2002;99(9):6328-6333.
 18. Cideciyan AV, Jacobson SG, Aleman TS, et al. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proc Natl Acad Sci USA*. 2005; 102(14):5233-5238.
 19. White DA, Hauswirth WW, Kaushal S, Lewin AS. Increased sensitivity to light-induced damage in a mouse model of autosomal dominant retinal disease. *Invest Ophthalmol Vis Sci*. 2007;48(5): 1942-1951.
 20. Heckenlively JR, Rodriguez JA, Daiger SP. Autosomal dominant sectoral retinitis pigmentosa: two families with transversion mutation in codon 23 of rhodopsin. *Arch Ophthalmol*. 1991;109(1): 84-91.
 21. Jacobson SG, Kemp CM, Sung CH, Nathans J. Retinal function and rhodopsin levels in autosomal dominant retinitis pigmentosa with rhodopsin mutations. *Am J Ophthalmol*. 1991;112(3):256-271.
 22. Fishman GA, Stone EM, Gilbert LD, Kenna P, Sheffield VC. Ocular findings associated with a rhodopsin gene codon 58 transversion mutation in autosomal dominant retinitis pigmentosa. *Arch Ophthalmol*. 1991;109(10):1387-1393.
 23. Stone EM, Kimura AE, Nichols BE, Khadivi P, Fishman GA, Sheffield VC. Regional distribution of retinal degeneration in patients with the proline to histidine mutation in codon 23 of the rhodopsin gene. *Ophthalmology*. 1991;98(12):1806-1813.
 24. Cideciyan AV, Hood DC, Huang Y, et al. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci USA*. 1998;95(12):7103-7108.
 25. Berson EL, Rosner B, Weigel-DiFranco C, Dryja TP, Sandberg MA. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. *Invest Ophthalmol Vis Sci*. 2002;43(9): 3027-3036.
 26. Iannaccone A, Man D, Waseem N, et al. Retinitis pigmentosa associated with rhodopsin mutations: correlation between phenotypic variability and molecular effects. *Vision Res*. 2006;46(27): 4556-4567.
 27. Massof RW, Finkelstein D. Two forms of autosomal dominant primary retinitis pigmentosa. *Doc Ophthalmol*. 1981;51(4):289-346.
 28. Fishman GA, Alexander KR, Anderson RJ. Autosomal dominant retinitis pigmentosa: a method of classification. *Arch Ophthalmol*. 1985;103(3):366-374.
 29. Lyness AL, Ernst W, Quinlan MP, et al. A clinical, psychophysical, and electroretinographic survey of patients with autosomal dominant retinitis pigmentosa. *Br J Ophthalmol*. 1985;69(5):326-339.
 30. Kemp CM, Jacobson SG, Faulkner DJ. Two types of visual dysfunction in autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1988;29(8):1235-1241.
 31. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res*. 1998;17(2):175-205.
 32. To K, Adamian M, Berson EL. Histologic study of retinitis pigmentosa due to a mutation in the RP13 gene (PRPC8): comparison with rhodopsin Pro23His, Cys110Arg, and Glu181Lys. *Am J Ophthalmol*. 2004;137(5):946-948.
 33. Li ZY, Jacobson SG, Milam AH. Autosomal dominant retinitis pigmentosa caused by the threonine-17-methionine rhodopsin mutation: retinal histopathology and immunocytochemistry. *Exp Eye Res*. 1994;58(4):397-408.
 34. To K, Adamian M, Berson EL. Histopathologic study of variation in severity of retinitis pigmentosa due to the dominant rhodopsin mutation Pro23His. *Am J Ophthalmol*. 2002;134(2):290-293.
 35. Huang Y, Cideciyan AV, Papastergiou GI, et al. Relation of optical coherence tomography to microanatomy in normal and rd chickens. *Invest Ophthalmol Vis Sci*. 1998;39(12):2405-2416.
 36. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Hum Mol Genet*. 2003;12(9):1073-1078.
 37. Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci USA*. 2005; 102(17):6177-6182.
 38. Aleman TS, Cideciyan AV, Sumaroka A, et al. Inner retinal abnormalities in X-linked retinitis pigmentosa with RPRG mutations. *Invest Ophthalmol Vis Sci*. 2007;48(10):4759-4765.
 39. Weinberger AW, Lappas A, Kirschkamp T, et al. Fundus near infrared fluorescence correlates with fundus near infrared reflectance. *Invest Ophthalmol Vis Sci*. 2006;47(7):3098-3108.
 40. Keilhauer CN, Delori FC. Near-infrared autofluorescence imaging of the fundus: visualization of ocular melanin. *Invest Ophthalmol Vis Sci*. 2006;47(8):3556-3564.
 41. Cideciyan AV, Swider M, Aleman TS, et al. Reduced-illumination autofluorescence imaging in ABCA4-associated retinal degenerations. *J Opt Soc Am A*. 2007;24(5):1457-1467.
 42. Paskowitz DM, LaVail MM, Duncan JL. Light and inherited retinal degeneration. *Br J Ophthalmol*. 2006;90(8):1060-1066.
 43. Jacobson SG, Voigt WJ, Parel JM, et al. Automated light- and dark-adapted perimetry for evaluating retinitis pigmentosa. *Ophthalmology*. 1986;93(12):1604-1611.
 44. Jacobson SG, Yagasaki K, Feuer WJ, Román AJ. Interocular asymmetry of visual function in heterozygotes of X-linked retinitis pigmentosa. *Exp Eye Res*. 1989;48(5):679-691.
 45. Aleman TS, Cideciyan AV, Volpe NJ, Stevanin G, Brice A, Jacobson SG. Spinocerebellar ataxia type 7 (SCA7) shows a cone-rod dystrophy phenotype. *Exp Eye Res*. 2002;74(6):737-745.
 46. Roman AJ, Schwartz SB, Aleman TS, et al. Quantifying rod photoreceptor-mediated vision in retinal degenerations: dark-adapted thresholds as outcome measures. *Exp Eye Res*. 2005;80(2):259-272.
 47. Jacobson SG, Cideciyan AV, Sumaroka A, et al. Remodeling of the human retina in choroideremia: rab escort protein 1 (REP-1) mutations. *Invest Ophthalmol Vis Sci*. 2006;47(9):4113-4120.
 48. Curcio C, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292(4):497-523.
 49. Jacobson SG, Sumaroka A, Aleman TS, Cideciyan AV, Danciger M, Farber DB. Evidence for retinal remodeling in retinitis pigmentosa caused by PDE6B mutation. *Br J Ophthalmol*. 2007;91(5):699-701.
 50. Jacobson SG, Cideciyan AV, Aleman TS, et al. RDH12 and RPE65, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. *Invest Ophthalmol Vis Sci*. 2007;48(1):332-338.
 51. Jacobson SG, Cideciyan AV, Aleman TS, et al. Leber congenital amaurosis caused by RPRGRI1 mutation shows treatment potential. *Ophthalmology*. 2007;114(5):895-898.

52. Grover S, Fishman GA, Anderson RJ, Alexander KR, Derlacki DJ. Rate of visual field loss in retinitis pigmentosa. *Ophthalmology*. 1997;104(3):460–465.
53. Cideciyan AV, Aleman TS, Jacobson SG, et al. Centrosomal-ciliary gene CEP290/NPHP6 mutations result in blindness with unexpected sparing of photoreceptors and visual brain: implications for therapy of Leber congenital amaurosis. *Hum Mutat*. 2007;28(11):1074–1083.
54. Humayun MS, Prince M, de Juan E Jr, et al. Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 1999;40(1):143–148.
55. Tsang SH, Gouras P, Yamashita CK, et al. Retinal degeneration in mice lacking the gamma subunit of the rod cGMP phosphodiesterase. *Science*. 1996;272(5264):1026–1029.
56. Huang Y, Cideciyan AV, Aleman TS, et al. Optical coherence tomography (OCT) abnormalities in rhodopsin mutant transgenic swine with retinal degeneration. *Exp Eye Res*. 2000;70(2):247–251.
57. Ren JC, Stubbs EB Jr, Matthes MT, et al. Retinal degeneration in the nervous mutant mouse. IV. Inner retinal changes. *Exp Eye Res*. 2001;72(3):243–252.
58. Frederick JM, Krasnoperova NV, Hoffmann K, et al. Mutant rhodopsin transgene expression on a null background. *Invest Ophthalmol Vis Sci*. 2001;42(3):826–833.
59. Marc RE, Jones BW, Watt CB, Strettoi E. Neural remodeling in retinal degeneration. *Prog Retin Eye Res*. 2003;22(5):607–655.
60. Weiland J, Fink W, Humayun M, et al. Progress towards a high-resolution retinal prosthesis. *Conf Proc IEEE Eng Med Biol Soc*. 2005;7:7373–7375.
61. Merabet LB, Rizzo JF 3rd, Pascual-Leone A, Fernandez E. ‘Who is the ideal candidate?’: decisions and issues relating to visual neuroprosthesis development, patient testing and neuroplasticity. *J Neural Eng*. 2007;4:S130–S135.
62. Bi A, Cui J, Ma YP, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron*. 2006;50(1):23–33.