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

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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 T17M-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. Scotomatus 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 laminar organization (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 hyporeflective 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

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 vitro6–9 and in animals with rbo mutations.10–19 What do we know about human RHO-ADRP? More than 100 mutations in RHO cause ADRP1,3,5 (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-ADRP:24 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.31,52 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-ADRP35 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

<table>
<thead>
<tr>
<th>Family (Mutation), Patient</th>
<th>Age(y)/Sex</th>
<th>Visual Acuity (RE, LE)*</th>
<th>Refraction†</th>
<th>Kinetic Visual Field Extent (V-ic)‡</th>
<th>ERG Amplitude§</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rod</td>
<td>Cone Flicker</td>
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<td>Class A RHO-ADRP</td>
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<tr>
<td>P1</td>
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<td>20/20</td>
<td>+3.75</td>
<td>79</td>
<td>25</td>
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</table>

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-ic 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 μV; cone flicker = 172 μV); 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.35,36,38 Lateral sampling density of the FD-OCT scans was 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.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 scleral 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 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 100 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 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 scleral boundary of the INL or the single hyporeflective layer continuous with the INL.35,36,38
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.1-8

**Histology in Mice**

Transgenic {\textit{mro}'''' T17M mice (H17M; age range, 4-6 months, \( n = 4 \))18 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 \( \mu \)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. (\( F (\text{family}) 1, P (\text{patient}) 1, \)) 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 coned-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 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...
A 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 (F2,P1, 104 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 non-detectable 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. In seven other patients with later-stage disease, retinal thickness was abnormally thick at most locations in all patients (Fig. 2B).

**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 ± 2SD) for retinal thickness (n = 27, ages 5–58) and inner retina (n = 14, ages 5–58). Insets: schematic location of the scans.
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 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 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.\textsuperscript{39–41} Class A patient F1,P1 showed a central ellipsoid region of homogeneously 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.\textsuperscript{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 retina\textsuperscript{48} (Fig. 4D). Of interest, the superior region with the most abnormal NIR-AF signal in the class B patient corresponded to the normal “rod hotspots”\textsuperscript{48} a small region of highest rod density (Fig. 4D).

Further Dissecting the Laminopathy in \textit{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, (mrho\textsuperscript{m} / Rho\textsuperscript{m})\textsuperscript{49} 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 μm 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 Laminopathy in the hT17M Rho Transgenic Mouse Retina

Do the laminar abnormalities in \textit{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 for wild-type mouse rhodopsin (Mrho\textsuperscript{17M} / rhodopsin\textsuperscript{m})\textsuperscript{50} Histologic sections of retinal tissue from 4-month-old hT17M mice revealed abnormal 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.

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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 hyporeflectivity 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,\textsuperscript{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 mutation\textsuperscript{19} and X-linked RP caused by RPGR mutations.\textsuperscript{38} Other human retinal degenerations with documented laminopathy include Leber congenital

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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 hyporeflective 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 hyporeflective layer that is continuous with the INL from the more central retina. Shaded areas: normal limits (mean ± 2SD; n = 9, age range, 15–63).
amaurosis\textsuperscript{36,50,51} and choroideremia.\textsuperscript{47} Major rod receptor losses and retinal remodeling in class A \textit{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 \textit{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 (\(\sim 10\) to \(20^\circ\) or \(3\)–\(6\) mm) of RPE abnormalities was observed with AF imaging in the region where normal retina shows highest rod densities.\textsuperscript{48} This suggests that RPE cell loss or depigmentation is occurring secondary to major rod photoreceptor loss. Although midperipheral scotomas (\(\sim 30^\circ\)–\(60^\circ\) or \(10\)–\(20\) mm) are the traditional hallmark of RP,\textsuperscript{52} it is intriguing that we found evidence of severe disease more centrally in the class B \textit{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 \(\sim 4\) 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 annulus\textsuperscript{48} in class B, with possibly a different natural history of

\begin{figure}[h]
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\caption{Detailed laminar structure of the superior retina in \textit{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 \textit{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. \(\star\) 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.}
\end{figure}
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 rbo mutant model of Class B. 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. The results from the transition zones in class B superior retina taken together with histopathology in the hT17M rbo 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. 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. 1; Ref. 57, Fig. 1; Ref. 58, Fig. 1; Ref. 59, Fig. 7). Vitrareal to the apparently single nuclear layer is an abnormally thick hyperreflective layer with uncertain morphologic basis. One contributor to this increased thickness may be epi-只怕 membrane, a common finding in RP. Detection of the ganglion cell layer within this thickened tissue is a relevant future translational issue for treatment with visual prosthetic devices 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. 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.

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**References**

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