Inner and Outer Retinal Changes in Retinal Degenerations Associated With *ABCA4* Mutations

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PURPOSE. To investigate in vivo inner and outer retinal microstructure and effects of structural abnormalities on visual function in patients with retinal degeneration caused by *ABCA4* mutations (*ABCA4*-RD).

METHODS. Patients with *ABCA4*-RD ($n = 45$; age range, 9–71 years) were studied by spectral-domain optical coherence tomography (OCT) scans extending from the fovea to 30° eccentricity along horizontal and vertical meridians. Thicknesses of outer and inner retinal laminae were analyzed. Serial OCT measurements available over a mean period of 4 years (range, 2–8 years) allowed examination of the progression of outer and inner retinal changes. A subset of patients had dark-adapted chromatic static threshold perimetry.

RESULTS. There was a spectrum of photoreceptor layer thickness changes from localized central retinal abnormalities to extensive thinning across central and near midperipheral retina. The inner retina also showed changes. There was thickening of the inner nuclear layer (INL) that was mainly associated with regions of photoreceptor loss. Serial data documented only limited change in some patients while others showed an increase in outer nuclear layer (ONL) thinning accompanied by increased INL thickening in some regions imaged. Visual function in regions both with and without INL thickening was describable with a previously defined model based on photoreceptor quantum catch.

CONCLUSIONS. Inner retinal laminar abnormalities, as in other human photoreceptor diseases, can be a feature of *ABCA4*-RD. These changes are likely due to the retinal remodeling that accompanies photoreceptor loss. Rod photoreceptor-mediated visual loss in retinal regions with inner laminopathy at the stages studied did not exceed the prediction from photoreceptor loss alone.

Keywords: retinal remodeling, optical coherence tomography, Stargardt disease

The primary insult in most hereditary retinal degenerations (RDs) is directed at the level of the photoreceptors or retinal pigment epithelium (RPE) cells.^{1,2} One of the more common molecular causes of RD is mutations in the *ABCA4* gene, which encodes ABCR, a photoreceptor-specific adenosine triphosphate (ATP)-binding cassette transporter.^{3–5} *ABCA4* is expressed in rod and cone outer segments^{6,7} and acts as an active transporter of all-*trans*-retinal (atRAL) from the lumen to the cytoplasmic leaflet of disc membranes.⁸ Mutations in *ABCA4* reduce the transport activity and result in the formation of potentially toxic bisretinoid adducts of atRAL within photoreceptors and/or the RPE.^{9–11} Either due to primary photoreceptor toxicity and stress or due secondarily to loss of RPE support, *ABCA4*-RD retinas show progressive degeneration.¹² Current therapeutic directions involve attempts to increase *ABCA4* transport function via gene augmentation therapy, or replace missing RPE cells in order to slow down or delay the natural history of RD or restore vision in *ABCA4*-RD (ClinicalTrials.gov numbers, NCT01367444, NCT01345006). There are also planned studies to reduce the rate of toxic

bisretinoid accumulation by modulating the visual cycle (e.g., see Ref. 13).

Potential treatments for *ABCA4*-RD directed to the rescue of photoreceptor function could be obstructed by abnormalities in the transmission of visual signals through the inner retina to the brain. Histological evidence from eye donors and animal models of RD support the hypothesis that degeneration of photoreceptors results in reactive and regressive changes in the remaining retinal neurons and glia (e.g., see Refs. 14–17). A recent report, based on histopathology in a postmortem eye donor with *ABCA4*-RD, suggested retinal remodeling in a severe retina-wide degeneration¹⁸; however, the remodeling consequences of other stages of *ABCA4*-RD are not known.

Availability of high-resolution cross-sectional imaging methods has made it possible to measure thickness of the inner and outer retinal lamina in living patients. We have previously shown abnormal thickening of inner retinal layers in RD patients with different molecular causes and proposed that these are likely signs of early reactive retinal remodeling secondary to photoreceptor degeneration.^{19–24} Similar studies have not been performed in *ABCA4*-RD to date. The current

study examines a cohort of patients with different disease severities of *ABCA4*-RD using cross-sectional imaging methods to evaluate inner retinal laminar changes. Further, longitudinal changes in outer and inner retinal layers are studied in patients with serial data collected over years. Finally, the question is asked whether there are detectable differences in visual dysfunction in regions with inner retinopathy compared to regions with photoreceptor loss only.

METHODS

Subjects

There were 45 patients with *ABCA4* mutations, representing 38 different families (Table). All patients underwent a complete eye examination. Normal subjects were included for optical coherence tomography (OCT; $n = 10$; age range, 8–45 years) and chromatic dark-adapted static perimetry ($n = 10$, age range, 15–57 years). Informed consent was obtained from all subjects. Procedures adhered to the Declaration of Helsinki and were approved by the institutional review board.

Imaging Studies

Retinal cross sections were obtained with OCT. Data collection at the most recent visit of all patients used a spectral-domain (SD) OCT system (RTVue-100; Optovue, Inc., Fremont, CA). In order to understand the natural history of retinal structural change, data from an earlier visit were used in a subset of 30 patients (Table); the earlier visit in 7 of 30 patients antedated SD-OCT, and the data were obtained with a time-domain (TD) OCT instrument (OCT3; Carl Zeiss Meditec, Dublin, CA). Postacquisition data analysis was performed with custom programs (MatLab 7.5; MathWorks, Natick, MA). Our OCT techniques have been published.^{20,25,26} In brief, scans were acquired along the horizontal and vertical axes extending to 30° from the fovea. Longitudinal reflectivity profiles (LRPs) making up the scans were aligned manually by straightening the major RPE reflection. Individual scans were digitally stitched to form 60°-wide horizontal or vertical scans centered on the fovea. The outer nuclear layer (ONL), inner nuclear layer (INL), and the combined thickness (termed GCC, ganglion cell complex²⁷) of the inner plexiform layer, ganglion cell layer, and nerve fiber layer (IPL+GCL+RNFL) located between the RPE and inner limiting membrane (ILM) were segmented manually by considering minima and maxima of backscatter signal amplitude, as well as the first derivative of the backscatter along the z -axis. Inner retinal thickness was defined as the distance from ILM to the vitreal boundary of outer plexiform layer (OPL).^{20,24,26} In many patients, there were transition regions surrounding central retinal atrophy, where there was a lamination change from one to two outer hyporeflective layers. In all these cases, the single outer hyporeflective layer was laterally continuous with the INL defined in normally laminated neighboring regions. A simple measure of the severity of outer retinal disease was defined in order to compare and contrast individual patients. This measure was the extent (radius) of ONL abnormality defined as the average retinal distances from the fovea along the temporal, superior, and inferior meridians where the ONL thickness reaches the lower limit of normal. Nasal retina was excluded from the ONL abnormality extent because of the complexity due to the parapapillary sparing commonly observed in *ABCA4*-RD.²⁸

It is now well known that a broad hyperscattering region originating from the Henle fiber layer (HFL) can appear scleral to the OPL and complicate measurements of ONL thickness.

Visibility of this HFL layer changes with the angle of incidence of imaging rays.^{29,30} As we previously described,³¹ our choice of OPL/ONL boundary is vitreal to the HFL reflection, and our definition of ONL thickness should include the anatomical layers encompassing both photoreceptor nuclei and HFL. The current study did not include data that would allow a reliable estimate of the HFL sublayer. Ideally, future work should include multiple OCTs obtained at each location by varying the angle of OCT beam incidence such that the HFL sublayer can be quantified.

Longitudinal OCT data available in a subset of patients (Table) were used to determine changes to INL and ONL thickness occurring over time. In order to determine the significance of thickness differences measured, intravisit repeatability of our INL and ONL measures was estimated. For this purpose, two *ABCA4*-RD patients (P17, P33) were chosen as representatives for the milder phenotypes (central ONL loss and normal INL thickness) and two patients (P5, P13) as representatives of the more severe phenotype (larger extent of ONL loss and substantial INL thickening). Inner nuclear layer and ONL thicknesses were measured from two independently acquired OCT scans across the horizontal meridian through the fovea obtained at the same (most recent) visit. Signed repeatability differences were obtained as a function of distance from the fovea for ONL and INL. Qualitatively, variation estimates for the ONL and INL appeared similar, and a t -test performed between ONL residuals and INL residuals showed no significant ($P > 0.05$) difference. For simplicity of presentation, ONL and INL residuals were combined to define confidence intervals of significant change.

Reduced-illumination autofluorescence imaging (RAFI) of the macular region was performed with short-wavelength (SW) illumination³² and a confocal scanning laser ophthalmoscope (HRA2 or Spectralis; Heidelberg Engineering, Dossenheim, Germany), mainly to illustrate the location of the OCT scans in some patients.

Dark-Adapted Chromatic Thresholds

Two-color dark-adapted static threshold perimetry was used to quantify rod-mediated visual function. Rod mediation was determined by comparison of sensitivities with a 500-nm stimulus to results using a 650-nm target. Sensitivity measurements were made at 2° intervals (1.7°-diameter target; 200-ms-duration stimulus) along the horizontal meridian in the temporal retina from 4° to 30°. Rod sensitivity loss was obtained by subtraction from mean normal using locus-specific normal data and expressed in log units.³³

The relationship between rod sensitivity and photoreceptor structure was quantified in retinal regions with and without INL thickening. An assessment of the structure-function relationship was performed eccentric to the foveal area and along the horizontal meridian in the temporal retina (4°–30°). The relationship of ONL thinning and rod sensitivity loss was compared to a previously developed model.^{26,34}

RESULTS

Outer and Inner Retinal Abnormalities in *ABCA4*-RD

There is a spectrum of disease phenotypes in *ABCA4*-RD ranging from mainly maculopathy to retina-wide degeneration.^{12,35–37} In vivo microstructure by OCT has demonstrated retinal structural changes in patients with *ABCA4*-RD; in some cases outer retinal photoreceptor layers have been quantified.^{28,32,36,38–40} Inner retinal abnormalities have not been

TABLE. Characteristics of the *ABCA4*-Related Retinal Disease Patients

Patient	Age at Visits, y	Sex	Allele 1	Allele 2	Previous Report*†
P1	9, 12	M	<i>E341G</i>	F608I	
P2	9, 15	M	R681X	C2150Y	P28*
P3‡	12	M	N965S	W821R	P1†
P4	13, 16	M	V256V	T1526M	P21*, P15†
P5	14, 20	F	W1408R	IVS40+5 G>A	P49*
P6‡	16	F	V989A	IVS28+5 G>T	P17†
P7‡	16	M	N965S	W821R	P18†
P8	18, 20	F	Y362X	IVS38-10 T>C	
P9‡	18	F	V989A	IVS28+5 G>T	
P10	18, 22	M	G1961E	R1129L	P3†
P11	20	M	R1640Q	c.5174_5175insG	
P12‡	20	M	G1961E	G1961E/P68L	
P13	22, 25	M	G863A	IVS35+2 T>C	P20†
P14	22, 24	F	G1961E	R152X	P12*, P21†
P15‡	23	M	G1961E	G1961E/P68L	
P16	25, 27	M	G1961E	R152X	P11*
P17	26, 32	F	L1940P	R1129L	P64*
P18	27, 34	F	<i>R1925G</i>	A1038V/L541P	
P19	27, 29	M	c.4530_4531insC	R1705Q	P52*, P5†
P20	28, 30	F	G1961E	A1038V/L541P	P23†
P21	31, 35	M	T1019M	G1961E	P34*
P22‡	32, 37	M	P1486L	Deletion of exon 7	P25†
P23	33, 35	M	G863A	R1108C	P29*, P6†
P24	34, 37	F	IVS40+5 G>A	V935A	P32*, P7†
P25	34	M	G1961E	§	P8†
P26	37, 43	F	C54Y	G863A	P4*
P27	39, 44	F	G863A	C1490Y	P30*, P26†
P28	40	M	G1961E	C54Y	P7*, P10†
P29	41	F	IVS38-10 T>C	E1087D	P59*
P30‡	43, 47	F	G1961E	V256V	P23*, P11†
P31‡	47, 51	F	P1486L	Deletion of exon 7	
P32	47	M	Y245X	Y245X	P20*
P33‡	48, 51	F	G1961E	V256V	P22*, P12†
P34	48, 50	F	c.3208_3209insTG	IVS40+5 G>A	
P35	50, 54	M	V1433I	L2027F	P50*
P36‡	52, 55	F	T1526M	R2030Q	P55*, P28†
P37	53, 59	F	G1961E	P1380L	P47*, P13†
P38‡	53, 61	M	L1940P	IVS40+5 G>A	P61*
P39	58	M	D600E	R18W	P2*, P14†
P40	59, 62	M	E1122K	G1961E	P44*
P41	59, 62	F	R1640Q	G1961E	P58*
P42‡	62	F	T1526M	R2030Q	P54*
P43‡	64, 68	M	L1940P	IVS40+5 G>A	P62*
P44	68	F	R1108C	IVS40+5 G>A	P42*
P45	71	F	IVS38-10 T>C	§	

Novel variants are bold and italicized.

* Patient previously reported in Cideciyan et al.¹²

† Patient previously reported in Cideciyan et al.⁸⁶

‡ Indicates sibling pairs: P3 and P7, P6 and P9, P12 and P15, P22 and P31, P30 and P33, P36 and P42, P38 and P43.

§ Second allele not yet identified.

studied quantitatively to date in *ABCA4*-RD. In contrast, there has been quantitative documentation of inner retinal abnormalities in many molecular forms of retinitis pigmentosa (RP), Leber congenital amaurosis, and other RDs in which it has been suggested that such findings can be used as a biomarker for retinal remodeling (e.g., see Refs. 19–24, 41–43).

The retinal laminae and their thicknesses in our cohort of 45 patients with *ABCA4*-RD were studied (Table). A cross-sectional OCT image of a 24-year-old normal subject shows the foveal depression and the alternating hyper- and hyposcattering layers. The outer (photoreceptor) nuclear layer (ONL) is highlighted for visibility, and the inner retina proximal to the ONL is shown (Fig. 1A, upper). The location of the OCT scan

of the normal subject is shown superimposed on SW-RAFI, which represents the distribution of the fluorescent signal dominated by RPE lipofuscin. Normally, there is relatively reduced SW-RAFI signal at the center of the macula due to absorption of SW light by macular pigment and higher signal at eccentricities farther from the center.³² An 18-year-old *ABCA4*-RD patient, P9, illustrates photoreceptor disease limited to the central retina with thinning and loss of ONL but normal ONL beyond an eccentricity of 5° (Fig. 1A, middle). The inner retina does not appear substantially different from that of the normal. The adjacent SW-RAFI image shows a central region of reduced RPE lipofuscin surrounded by hyperautofluorescent ring and flecks. More extensive disease is illustrated in the OCT and

FIGURE 1. Outer and inner retinal abnormalities in *ABCA4*-RD. **(A)** Cross-sectional OCT images along the horizontal meridian through the fovea in a normal subject are compared with those of two patients with *ABCA4*-RD. Different degrees of outer nuclear layer (ONL, highlighted in blue) loss are illustrated. Vitread to the ONL is the inner retina. Scan line (white) is shown for each OCT on a short-wavelength reduced-illumination autofluorescence image from the same subjects (right). Image intensities are scaled for visibility of features. **(B)** Outer retinal disease severity of each patient is ranked by average ONL abnormality extent. Age of each patient is shown below the ONL abnormality. Arrows above the bars denote data from the two patients shown below. **(C, D)** Representative ONL and inner retina thickness profiles from two patients with different degrees of ONL thickness reduction compared with normal (gray bands; mean $\pm$ 2SD). **(E, F)** Locus-specific inner retina thickness fraction of normal mean plotted as a function of ONL thickness fraction in *ABCA4*-RD patients (black symbols) compared to data from normal subjects (gray symbols). Horizontal gray dashed line: upper limit of normal inner retina fraction (mean + 2SD). Vertical gray dashed line: lower limit of normal ONL fraction (mean - 2SD). Red symbols in **(E)** and **(F)** represent the data from patients shown in **(C)** and **(D)**, respectively.

autofluorescence images of P13, age 25 (Fig. 1A, lower). Most of the ONL in the OCT is detectable but abnormally reduced in thickness; the inner retina appears particularly thickened in the temporal parafovea. There is also severe macular RPE disease by SW-RAFI with a central region of reduced RPE lipofuscin surrounded by a heterogeneous region of hypo- and hyperautofluorescence across the macula and beyond.

Among the 45 *ABCA4*-RD patients, 17 (37.8%) had ONL abnormalities that did not extend beyond a radius of 12° from the fovea; 10 patients (22.2%) showed ONL thinning that extended beyond 12° but less than or equal to an 18° radius; 9 patients (20%) showed ONL abnormalities larger than 18° but less or equal to 27° radius; and in 9 patients (20%), there were ONL abnormalities extending beyond 27° or across the full length (30°) of the scanned region (Fig. 1B). There was no significant relationship between patient age and ONL abnormality extent in this cohort of patients ($r^2 = 0.05$, $P = 0.15$; Fig. 1B, lower).

Results from two patients exemplify the outer and inner retinal thicknesses measured at two extremes of the disease severity spectrum (Fig. 1B, arrows). The ONL and inner retinal thickness profiles for P23 at age 35 illustrate *ABCA4* disease with involvement of only the central macula (Fig. 1C). Outer nuclear layer abnormality is found up to ~6° to 7° extent from the fovea, and inner retinal thickness is within normal limits. Patient 8 at age 20 illustrates more severe *ABCA4* disease in which ONL thickness is abnormal at all eccentricities measured along both horizontal and vertical meridians. Inner retina shows abnormal thickening at nearly all locations measured (Fig. 1D). These examples implied that greater photoreceptor disease was associated with greater inner retinal thickening. To better understand the relationship between ONL and inner retina in *ABCA4*-RD, we plotted the inner retinal thickness as a function of ONL thickness in patients with different extents of ONL abnormality (black symbols; $n = 16$ patient subset); the patient results are plotted in relation to data from normal subjects (gray symbols; $n = 10$; Figs. 1E, 1F). For these plots, we normalized the thickness measures by the respective mean normal value at each retinal location in order to account for large changes in thickness that occur across normal retinas. Retinal regions with normal ONL thickness had mostly normal inner retinal thickness as exemplified by the results of P23 (Fig. 1E, red symbols). Retinal regions with abnormal ONL thickness could also have normal inner retinal thickness as in P23 (Fig. 1E, red symbols) and in some of the loci in P8 (Fig. 1F, red symbols). The majority of loci in P8 with abnormal ONL thickness were associated with abnormally thickened inner retina (Fig. 1F, red symbols).

Contribution of the Inner Nuclear Layer to Inner Retinal Abnormalities

To better understand the source of the inner retinal abnormalities in *ABCA4*-RD, we considered the two major subdivisions of this layer: Distally near the ONL is the INL, whereas proximally toward the vitreous is what has been termed the ganglion cell complex (GCC), consisting of the inner plexiform layer (IPL), ganglion cell layer (GCL), and retinal nerve fiber layer (RNFL).^{24,27} In an OCT scan from the temporal retina of a representative normal subject, the ONL, INL, and GCC are labeled (Fig. 2A, upper). Qualitative inspection of OCT scans from three representative patients showed a range of results for INL thickness from normal to abnormally thick; the GCC layer appeared mostly similar to normal (Fig. 2A). To quantitatively characterize this impression, GCC and INL thicknesses were measured in all patients, and a representative subset ($n = 20$) is plotted (Fig. 2B). Since there were substantially greater retinal loci showing INL thickening

(1902/6447 loci; 29.5%) compared to GCC thickening (897/6647 loci; 13.9%), further retinal structural analyses were performed only with INL and ONL thicknesses (Figs. 2C–F).

Given these observations of possible and definite INL thickening with ONL loss, we quantified the relationship of photoreceptor loss and INL thickening in *ABCA4*-RD. Inner nuclear layer and ONL thicknesses were quantified in both horizontal and vertical meridians in the entire cohort of 45 *ABCA4*-RD patients as normalized by locus-specific mean normal values; loci used in the analysis excluded the foveal region that normally lacks an INL.^{19,44} Normal limits of ONL and INL thickness ratios were determined (Fig. 2C; vertical dashed line, lower limit of ONL fraction; horizontal dashed line, upper limit of INL fraction). The majority of retinal loci in *ABCA4*-RD patients had normal INL thickness, and these loci were divided between those with normal ONL (2631/6447 loci; 40.8%) and those with abnormally reduced ONL (2048/6447 loci; 31.8%). Abnormally thick INL was associated mostly with retinal loci demonstrating abnormally reduced ONL (1720/6447 loci; 26.7%). It was rare (48/6447 loci; 0.7%) to observe abnormally thinned INL associated with normal or thinned ONL, or abnormally thickened INL associated with normal ONL thickness.

The unexplained overlapping of normal and *ABCA4*-RD data led us to examine INL and ONL relationships in individual patients. We found that there could be different associations within the same retina (Figs. 2D–F). Patient 23 (age 35) exemplifies patients that showed two of the relationships of ONL and INL: either no difference from normal thickness for ONL and INL, or reduced ONL but no associated INL thickening (Fig. 2D, upper left). Quantitation of INL and ONL thickness from P33 shows that ONL was reduced within a radius of ~5° from the fovea but normal at further eccentricities; INL was within normal limits throughout the profiles including the macular region with ONL thinning. Seventeen patients with an extent of ONL abnormality < ~11.5° showed patterns similar to that of P33 (Fig. 2D, upper right). Patient 22 (age 37) had three different associations. Some loci were within normal limits for ONL and INL; other regions had reduced ONL but normal INL; and there were regions with reduced ONL associated with thickened INL (Fig. 2E). Ten patients with ONL abnormal extent between ~18° and ~27° showed a pattern similar to that in P22 (Fig. 2E, upper right). Patient 1 (age 12) had reduced ONL associated with INL thickening at all loci sampled (Fig. 2F). Nine patients with ONL abnormality > 27° in extent showed results similar to those of P1 (Fig. 2F, upper right). The remaining nine patients represent the group with abnormal ONL extent between ~11.5° and 18° (data not shown). In this subset, three patients showed INL thickening in regions with ONL reduction, while six did not show INL thickening even though there was ONL reduction.

Inner and Outer Retinal Disease Progression: Longitudinal Data

The cross-sectional data we collected in *ABCA4*-RD showed two extremes: Those patients with only limited central retinal ONL loss had little or no INL thickening, while those with the greatest extent of ONL loss showed INL thickening across the sampled region. Longitudinal results in a subset of patients permitted the question to be asked whether progression from one to the other (or to intermediate patterns) could be detected.

A subset of 30 patients had serial data collected (mean interval, 4 years; range, 2–8; Table). Two representative examples show inner and outer disease documented over a 4- to 5-year interval (Fig. 3A). An OCT scan of the temporal retina of P21 at age 31 showed an ONL abnormality limited to

FIGURE 2. Inner nuclear layer (INL) thickness changes in relation to ONL thickness in *ABCA4*-RD. **(A)** Cross-sectional OCT images showing temporal retina along the horizontal meridian through the fovea. ONL (blue), INL (purple), and the ganglion cell complex (GCC, bracket) are labeled in a normal subject and in three *ABCA4*-RD patients with different degrees of ONL loss. Between arrows (in P19, P5) are regions with thickened INL. **(B)** INL and GCC thickness in a subset of 20 patients demonstrating that inner retinal abnormalities are mainly in the INL. Gray bands represent normal results (mean $\pm$ 2SD). **(C)** Locus-specific INL fraction plotted as a function of ONL fraction in *ABCA4*-RD patients (black dots) compared to data from normal subjects (gray dots). Horizontal dashed gray line: upper limit of normal INL fraction (mean + 2SD). Vertical

dashed gray line: lower limit of normal ONL fraction (mean $- 2SD$). (D-F) Groups of patients with different degrees of INL and ONL thickness abnormalities, and details of representatives from each group. (D) Detailed results of P33 with normal INL thickness as a representative of the group of 17 patients with ONL abnormality extent $< \sim 11.5^\circ$ (highlighted in red, upper right). (E) Detailed results of P22 showing a range of relationships between ONL and INL thickness from normal to ONL reduction and INL thickening. This patient is representative of the group of 10 patients with ONL abnormality extent larger than $\sim 16.5^\circ$ but less than $\sim 26.5^\circ$ (highlighted in red, upper right). (F) Detailed results of P1 with abnormally reduced ONL and abnormally thickened INL at almost all loci as a representative of the group of nine patients with ONL abnormality extent greater than $\sim 26.5^\circ$ (highlighted in red, upper right). Gray bands: normal limits (mean $\pm 2SD$). Arrow beneath ONL abnormality extent graphs (upper right) identifies the patient being illustrated in other panels of (C-E).

the central region. At age 35, there was very little structural change (Fig. 3A, left). Inner nuclear layer and ONL thicknesses were quantified along the temporal retina. The ONL, outside of the central loss, was retained, and the INL was within normal limits at both ages studied (Fig. 3A, lower left). An OCT of P26 at age 37 (Fig. 3A, right) shows ONL that is abnormally reduced but detectable throughout the scan; reduction is greater in the more central region. At age 42, ONL is further reduced centrally but still detectable at an eccentricity of $\sim 13^\circ$ from the fovea. The INL was within normal limits or showed regions of thickening at age 37; but at age 42, there was more prominent INL thickening, especially in the regions where ONL was no longer detectable. Inner nuclear layer and ONL thickness quantitation confirmed the observations made by visual inspection of the scans (Fig. 3A, lower right).

Study of available serial data in the temporal retina indicated that 18 of the 30 patients were similar to P21 in that there were no prominent thickness changes (group 1) in the interval (examples in Fig. 3B). The remaining 12 patients, however, were more similar to P26, and showed regions that changed (group 2) in the interval between visits (examples in Fig. 3C). Outer nuclear layer reduction was accompanied by INL thickening; some changes were more parafoveal in location, and others extended to within 3° of the edge of the 30° of temporal retinal scan length sampled (Fig. 3C). We asked whether there were factors contributing to this dichotomy of results. The average age at first visit was no different between the two groups (average ages 36.9 and 31.3 years for groups 1 and 2, respectively, $P = 0.34$), and the interval was also not different (3.72 and 3.75 years for groups 1 and 2, respectively, $P = 0.98$). The average ONL abnormality extent in the two groups at first visit was significantly different (12° and 19° for groups 1 and 2, respectively, $P = 0.012$). This suggests that patients in group 2 had more severe outer retinal disease phenotype in their first visits. Next, we asked whether the INL changes were associated with progression of the extent of ONL abnormality in the patients. The average ONL abnormality extent in group 1 at the later time point was 12.7° , and outer retinal structure in these patients was relatively preserved ($P = 0.06$). The average ONL abnormality extent in group 2 at the later time point was 24° , and it was different from the value at the earlier visit ($P = 0.002$). Taken together, the data suggest an association of photoreceptor loss and INL change. The microstructural retinal abnormalities and association of ONL thinning and INL thickening observed in the cross-sectional data were thus confirmed in some patients to represent stages of disease progression.

Visual Function and Outer-Inner Retinal Architecture in *ABCA4*-RD

An unanswered question in the literature about human inner laminopathy associated with outer retinal disease is whether INL thickening in an area of ONL abnormality is associated with greater dysfunction than expected from an idealized photoreceptor degeneration alone. In this series of *ABCA4*-RD patients, we asked this question using colocalized rod

sensitivity measurements and OCT parameters quantified horizontally along the temporal retina. Regional differences in the relationship between ONL and INL thickness in the same patient provided a further opportunity to compare structure and function. Structural data for ONL and INL thickness are shown for three of the patients (P2, P19, P13). The data included loci with ONL loss but no INL thickening, as well as loci with both ONL loss and INL thickening. There were also regions in which both ONL and INL were within normal limits (Fig. 4A). Bar plots permit a comparison of ONL thickness and colocalized rod sensitivity in the three patients (Fig. 4A, lower). Note that there is a factor of 2 difference along the logarithmic ordinates to compensate for the underlying prediction of sensitivity being proportional to ONL squared (see below). Thus bars of equal length indicate good agreement of the patient data with the idealized pure photoreceptor degeneration model.²⁶ For these three patients, there is qualitative agreement with the model for retinal regions showing abnormal INL thickening (green bars) as well as retinal regions with normal INL thickness (black bars).

Quantitative analyses of the relationship between rod sensitivity and photoreceptor structure were performed in rod-dominated extrafoveal retinal regions with and without inner laminopathy in 10 *ABCA4*-RD patients (Figs. 4B, 4C). In retinal degenerative diseases involving only the photoreceptors, the expected relationship between photoreceptor structure and visual function would be predicted to show proportionality between threshold sensitivity to light and the number of light quanta absorbed in photoreceptor outer segments. The latter would in turn be expected to be proportional to both the number of surviving photoreceptors and the length of their outer segments. To a first approximation, ONL thickness can be used as a surrogate metric for both the number of photoreceptors and outer segment length, and thus light sensitivity (in linear units) would be expected to be proportional to the square of ONL thickness.^{26,34,45} Both retinal loci with normal INL thickness (Fig. 4B) and retinal loci with abnormally thickened INL (Fig. 4C) showed a relationship of ONL thickness and rod sensitivity loss as predicted by the model of an idealized photoreceptor degeneration (χ^2 analyses; $P = 0.992$, $P = 0.9997$ for Figs. 4B, 4C, respectively). There were also no significant differences when considering residuals between patients' data and the model (Mann-Whitney rank sum test; $P = 0.122$). These results suggest that there was no evidence of an additional rod sensitivity loss associated with inner retinal remodeling.

DISCUSSION

Is There Evidence of Retinal Remodeling in *ABCA4*-RD?

An affirmative answer comes with caveats, based on the current study of a cohort of patients with a wide spectrum of disease severity. *ABCA4*-RD disease with ONL loss limited to the central retina was not accompanied by detectable inner retinal change. The definition of the disease, however, has

FIGURE 3. Longitudinal progression of retinal structural changes in *ABCA4*-RD. **(A)** Representative serial OCT images in two patients. P21, between ages 31 and 35 (*left*), did not show measurable change. P26, between ages 37 and 42 (*right*), had ONL reduction and INL thickening. Quantitation of INL and ONL thicknesses along the temporal retina for these two patients is shown (*lower*). Gray bands: normal limits (mean $\pm$ 2SD). **(B, C)** Magnitude of INL (purple traces) and ONL (blue traces) thickness changes in 12 patients as a function of eccentricity in temporal retina measured between two visits. In six of the patients (**B**), changes in the thickness of retinal structures were not different than test-retest variability, while in six other patients (**C**), there were substantial reductions of ONL and thickening of INL in certain regions. Light gray bands: intravisit test-retest variability of ONL and INL thickness estimated in *ABCA4*-RD patients.

FIGURE 4. Retinal structure and function in *ABCA4*-RD. **(A)** Data from three representative patients demonstrating colocalized measures of INL (first row) and ONL thickness (second and third rows) and rod sensitivity loss (RSL, fourth row) in the temporal retina as a function of eccentricity from fovea (F). Retinal regions corresponding to abnormally thick INL are highlighted in green. ONL thickness as a fraction of normal mean (third row) and colocalized RSL are shown in log units. Note that the factor of 2 in the scales of the ordinate corresponds to the predicted proportionality between sensitivity and ONL squared. The inset (top right) illustrates coverage in relation to retinal landmarks. Open circle: optic nerve head; filled circle: fovea. T, temporal retina; F, fovea. Gray bands: 2SD limits of normal. **(B, C)** Relationship between ONL thickness (as a fraction of normal

mean) and visual function (RSL) for temporal retina loci across the horizontal profile in 10 *ABCA4*-RD patients. Normal variability is described by an ellipse encircling the 95% confidence interval of a bivariate Gaussian distribution. *Dashed lines* delimit the region of uncertainty that results by translating the normal variability along the idealized model for pure photoreceptor degenerations. In these patients, loci with normal INL thickness (**B**) as well as those with thickened INL (**C**) remain mostly consistent with the zone predicted by the model.

widened with molecular progress and now includes not only autosomal recessive juvenile-onset hereditary macular dystrophy but also cone-rod dystrophy patients and some patients diagnosed with variants of RP (reviewed in Ref. 37). In the more severely affected *ABCA4*-RD patients with outer retinal abnormalities extending beyond a radius of $\sim 10^\circ$ from the fovea, there was definite evidence of inner retinal laminopathy. The structural changes corresponded to abnormal thickening of the inner retina at extrafoveal regions with ONL reduction, and resembled those we previously suggested to represent a biomarker of retinal remodeling in other human RDs (e.g., see Refs. 19–24, 41–43). After segmenting the OCT scans to subdivide the inner retina into INL and more vitreal laminae, it was evident that INL thickening was the main contributor to the inner retinal abnormalities.

Cross-sectional studies of the cohort of *ABCA4*-RD patients indicated that INL thickening was associated with ONL thinning. Serial data were used to try to determine whether the cross-sectional observations represented disease progression. A subset of patients showed no change in ONL or INL over the time periods studied. The natural history of *ABCA4*-RD patients has been explored in detail, and such data were consistent with a proposed model that showed a plateau phase of varying length and then an exponential decline in rod and cone function.¹² From such data, it is likely that the subset of patients without measurable structural change were on the plateau phase of their natural history. In contrast, there was another subset of patients that had thinning of ONL and thickening of INL in the interval between studies. These patients with disease progression were likely on the decline predicted by the model. Although it is not surprising that some patients undergo disease progression^{46–49} and lose photoreceptors (ONL), it was not known previously that this was accompanied by reactive abnormalities in the inner retinal structure detectable within the time period of serial observation. Further quantitative studies are required to determine how the rates of increasing inner retinal laminopathy relate to differences in rates of photoreceptor degeneration as evidenced by thinning ONL.

Visual Consequences of Retinal Remodeling in Retinal Degenerations and Specifically *ABCA4*-RD

In animal models of RD, there have been rare reports on the relationship between retinal structure and visual function in the presence of remodeling. In transgenic pig and rabbit models of Class A rhodopsin mutations causing autosomal dominant RP⁵⁰ there are visual function abnormalities related to the inner retinal cone circuitry that are substantially greater than the loss of photoreceptor function.^{51–54} Histological studies have shown evidence of postreceptoral remodeling and reprogramming in pigs^{55,56} and in rabbits.^{54,57} In *rd* and *rd10* mice, there has been evidence of retina-wide dysfunction beyond that explained by photoreceptor loss and consistent with extensive remodeling of the second-order neurons.^{58,59} Interestingly, however, a study of remnant cone photoreceptors within large patches of RD in *rd10* mice demonstrated retained presynaptic proteins needed for photoreceptor function.⁶⁰

In human RD, it has been demonstrated with electroretinography (ERG) that retina-wide postreceptoral dysfunction can be greater than that expected from receptor dysfunction;

these studies antedated availability of noninvasive measures of retinal laminar architecture by OCT.^{61,62} More recently, inner retinal laminopathy in RDs with loss of visual function has been reported (e.g., see Refs. 19–24, 41–43, 63, 64); and some of these diseases (at certain stages) show, by ERG parameters, unexpected inner retinal dysfunction for the amount of receptor dysfunction. Studies including ERG data, both cross-sectional and longitudinal, in Stargardt disease or *ABCA4*-RD have provided valuable information about disease staging or subclassification as well as prognostic indicators,^{12,35,46,65–67} but the focus has not been on inner retinal structure versus function.

In *ABCA4*-RD, colocalized structure–function relationships have been previously reported,^{32,68} but inner retinal structure has not been quantified, to our knowledge. To place the importance of making such measurements in a clinical therapeutic context, two major groups of treatment strategies are planned for *ABCA4*-RD depending on whether photoreceptors exist at the time of the intervention.⁶⁹ One group would aim to replace the photoreceptors that have completely degenerated at the time of the intervention and includes electronic chips, cell transplantation, and optogenetics.^{24,70–72} It has been a concern that retinal remodeling could potentially limit any visual improvement if the remaining postreceptoral circuitry is dysfunctional. The current work showed retinal remodeling in regions representing the latest stages of *ABCA4* disease. It was, however, not possible to estimate the visual function consequences ascribed to retinal remodeling since these regions had no photoreceptors and no vision to measure. The second group of treatment strategies would aim to maintain or improve the function of photoreceptors existing at the time of the intervention and includes gene augmentation,^{73–75} visual cycle modulators,^{13,76–80} and other nutritional and pharmacological approaches.^{81–84} Retinal remodeling could potentially affect the flow of signal from remnant photoreceptors to higher visual centers and thus limit the outcome of the treatment strategy. Our results provide evidence for detectable inner retinal laminopathy in regions with disease stages corresponding to partially retained photoreceptors, but there was no evidence of a major visual function consequence of such retinal remodeling.

The current study is the first for *ABCA4*-RD and for any form of RD to attempt a quantitative comparison of the structure–function relationship in retinal regions with a range of retained photoreceptors and a range of inner retinal abnormalities. The lack of major visual function consequence of the inner retinal laminopathy found here in *ABCA4*-RD suggests that circuitry between remnant photoreceptors and postreceptoral cells remains relatively intact. Or, a subset of remnant photoreceptors retain their functional connectivity individually, at least for some disease stages, even when surrounded by severe retinal remodeling secondary to the loss of neighboring photoreceptors. Alternatively, there are contributions from reactive synaptic changes as hypothesized in some animal models of RD and aging,⁸⁵ and such changes partially compensate for the circuitry changes secondary to remodeling. With any of these hypotheses, our results bode well for treatment strategies directed to the maintenance or improvement of remnant photoreceptors in *ABCA4*-RD.

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