The Structure and Function of the Macula in Patients with Advanced Retinitis Pigmentosa

Rita Vámos,^{1,2} Erika Tátrai,^{1,2} János Németh,¹ Graham E. Holder,^{3,4} Delia Cabrera DeBuc,⁵ and Gábor Márk Somfai¹

PURPOSE. To assess the structure and function of the macula in advanced retinitis pigmentosa (RP).

METHODS. Twenty-nine eyes of 22 patients with RP were compared against 17 control eyes. Time-domain optical coherence tomography (OCT) data were processed using OCTRIMA (optical coherence tomography retinal image analysis) as a means of quantifying commercial OCT system images. The thickness of the retinal nerve fiber layer (RNFL), ganglion cell layer and inner plexiform layer complex (GCL+IPL), inner nuclear layer and outer plexiform layer complex (INL+OPL), and the outer nuclear layer (ONL) were measured. Multifocal electroretinography (mfERG) was performed; two groups were formed based on the mfERG findings. Fourteen eyes had no detectable central retinal function (NCRF) on mfERG; detectable but abnormal retinal function (DRF) was present in the mfERG of the other 15 eyes.

RESULTS. The thickness of the ONL in the central macular region was significantly less in the NCRF eyes compared with that in both DRF eyes and controls. The ONL was significantly thinner in the pericentral region in both patient groups compared with that in controls, whereas the thickness of the GCL+IPL and INL+OPL was significantly decreased only in the NCRF eyes. The RNFL in the peripheral region was significantly thicker, whereas the thickness of the GCL+IPL and ONL was significantly thinner in both patient groups compared with that in controls.

CONCLUSIONS. The results are consistent with degeneration of the outer retina preceding inner retinal changes in RP. OCT image segmentation enables objective evaluation of retinal

structural changes in RP, with potential use in the planning of therapeutic interventions and conceivably as an outcome measure. (*Invest Ophthalmol Vis Sci.* 2011;52:8425–8432) DOI: 10.1167/iovs.11-7780

Retinitis pigmentosa (RP) is the most common inherited retinal dystrophy, with a worldwide prevalence of approximately 1:4000.¹ The disease is characterized by progressive night blindness and visual field constriction, a rod–cone pattern of electroretinographic (ERG) abnormality (if any ERG remains detectable) and characteristic degenerative retinal changes. Long-term visual prognosis in RP is difficult to predict: some patients maintain acceptable visual function until an advanced age; many do not. The initial degenerative changes occur in the photoreceptors, especially in the midperipheral retina where rod photoreceptor density is maximal. Cone cell death is probably consequent on rod photoreceptor death. As the disease progresses the macula may or may not become involved.²

The structure of the macula can be assessed objectively using optical coherence tomography (OCT).³ Commercially available OCT devices allow limited measurement of the retinal nerve fiber layer (RNFL) thickness around the optic disc along with the thickness of the retina in the macula. Quantitative data about the intraretinal structures can be observed with the use of OCT image processing softwares. Several authors have reported OCT thinning of the photoreceptor layer in patients with RP.^{4–7} However, there is little consistency emerging from studies of inner retinal structure.^{4–6,8–10}

Multifocal electroretinography (mfERG) is a noninvasive method that allows assessment of the spatial distribution of the function of central retinal cones. In advanced stages of RP mfERG represents only residual cone activity. The present study correlates the results of mfERG with OCT parameters that include retinal thickness measurements from OCT.

PATIENTS AND METHODS

Patients diagnosed with RP and who had received both OCT and mfERG at the same visit between November 2006 and March 2010 at the Department of Ophthalmology, Semmelweis University, Budapest, Hungary, were retrospectively reviewed. Exclusion criteria were the presence of any other ocular or optic nerve disease, including glaucoma, or of any systemic disease other than controlled hypertension. Exclusion criteria based on OCT imaging were the following: cystoid macular edema, with or without epiretinal membrane formation; a low signal strength (SS) of the OCT images (SS < 6); and foveal decentration (center point thickness SD > 10%). Twenty-nine eyes of 22 from 57 RP patients were included (16 males and 6 females, median age: 32 years; range: 14 to 63 years). Diagnostic criteria of RP included progressive night blindness and visual field constriction, a rod–cone pattern of ERG abnormality, atrophic optic discs, and intraretinal bone spicule pigmentary deposition (bilateral). Among the study subjects ten had sporadic, one had autosomal recessive, two had autosomal

From the ¹Department of Ophthalmology, Semmelweis University, Budapest, Hungary; the ²Moorfields Eye Hospital, London, United Kingdom; the ³Institute of Ophthalmology, University College London, London, United Kingdom; and the ⁴Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida.

²These authors contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Supported in part by a Juvenile Diabetes Research Foundation grant, National Eye Institute Grant P30-EY014801, an unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc., a Zsigmond Diabetes Foundation of the Hungarian Academy of Science (Budapest) grant, a National Institute for Health Research United Kingdom grant (GEH), Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital National Health Service Foundation Trust and University College London Institute of Ophthalmology, The Foundation Fighting Blindness (Columbia, MD), and the Alcon Research Institute.

Submitted for publication April 22, 2011; revised September 13, 2011; accepted September 14, 2011.

Disclosure: R. Vámos, None; E. Tátrai, None; J. Németh, None; G.E. Holder, None; D. Cabrera DeBuc, P; G.M. Somfai, None

Corresponding author: Gábor Márk Somfai, Semmelweis University, Department of Ophthalmology, 39 Mária Str., 1085 Budapest, Hungary; somfaigm@yahoo.com.

FIGURE 1. Time-domain OCT scan of a healthy macula (A) and the corresponding segmentation by OCTRIMA (B) after minor manual corrections in the segmentation. V, vitreous; Ch, choroids.

dominant, and one had X-linked RP. Eight patients had a positive family history but no definitive inheritance pattern could be established (either due to lack of information or the small number of relatives). Confirmatory mutational data were not available.

For the OCT control group 17 eyes from 17 age-matched controls were randomly selected from the normative database (median age: 31 years; range: 21 to 59 years). Eligibility criteria for control subjects were best-corrected Snellen visual acuity (VA) of 20/20 and the lack of any ophthalmic, neurologic, or systemic diseases. All control subjects gave informed consent and the study conformed to the tenets of the Declaration of Helsinki. No Institutional Review Board approval was required for the study.

OCT was performed using a time-domain (TD)-OCT device (Stratus OCT; Carl Zeiss Meditec, Dublin, CA). Each eye was scanned using the “macular thickness map” protocol, consisting of six radial scan lines centered on the fovea, each having a 6-mm transverse length. The OCT raw data were exported from the device and further processed with optical coherence tomography retinal image analysis (OCTRIMA), which is an interactive, stand-alone application for analyzing TD-OCT retinal images.^{11,12} Segmentation errors were manually corrected using the manual correction tool provided by OCTRIMA.

The thickness values for the RNFL, ganglion cell layer and inner plexiform layer complex (GCL+IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and the total retina were recorded for each eye in each Early Treatment Diabetic Retinopathy Study (ETDRS) region¹⁵ (see Fig. 1). It is important to note that OCTRIMA measures total retinal thickness between the vitreoretinal border (ILM) and the inner boundary of the second hyperreflective band, which has been attributed to the outer segment/retinal pigment epithelium (OS/RPE) junction, in agreement with histologic studies.^{14–16} Moreover, the sublayer labeled as ONL is actually enclosing the external limiting membrane and inner segment (IS), but in the standard 10- μ m resolution OCT image this thin membrane cannot be clearly visualized, making the segmentation of the IS difficult. Also, since there is no significant luminance transition between the GCL and the IPL, the outer boundary of the GCL layer is difficult to visualize in the image. Thus, a combined GCL+IPL layer is preferable. To obtain more precise measurements, the INL and OPL were collapsed and measured together for the analyses because the reproducibility of the layers taken separately is worse than that of the collapsed layer. In addition, the intraretinal layers in various eccentricities from the fovea were assessed by calculating the mean thickness of the layers for the central (R1), pericentral (R2–R5), and peripheral (R6–R9) ETDRS regions.

mfERG (RETI-scan; Roland Consult, Stasche & Finger GmbH, Wiesbaden, Germany) was recorded monocularly using ERG-jet electrodes and a 61-hexagon stimulus according to the guidelines of the International Society for Clinical Electrophysiology of Vision,¹⁷ with a 21-inch video stimulating display (CRT monitor, 75-Hz frame rate, cutoffs: 10–100 Hz) subtending 30° on either side of fixation. A narrow “X” was used for fixation, to cover as little of the central stimulus element as possible. Patients’ fixation was continuously monitored using a

camera system. Two recordings were obtained, each approximately 4 minutes in duration. Any large eye movements or fixation losses were rejected and the recording was repeated. The retinal area stimulated by the central hexagon was between 0 and 2.5°, by ring 2 between 2.5 and 8°, and by ring 3 between 8 and 15° eccentricity from the fovea on either side.¹⁸ Therefore, the central ETDRS subfield corresponds mainly to the central hexagon area on mfERG and the pericentral ETDRS subfield corresponds mainly to the second ring of hexagons,^{18,19} as shown in Figure 2.

Both trace array and ring presentation of first-order kernels were performed and evaluated. Since earlier work has shown that the retinal signal occurs within the first 60 ms after stimulation,^{20,21} each trace recording was divided into two epochs: a signal epoch between 15 and 75 ms and a noise epoch between 100 and 150 ms. Because signal to noise ratio (SNR) methods provide a better reliability to discriminate signal from noise in recordings from patients with retinitis pigmentosa, where traces are very attenuated, signal detection based on SNR was used.²² The root mean square (RMS) amplitude for each of these epochs was calculated as a measure of the magnitude for each epoch. The SNR was calculated by dividing the RMS amplitude of the signal epoch by the mean of the RMS amplitude of the noise epoch at each hexagon, providing further calculation according to eccentricities. The SNR would be close to 1 if a waveform contained no signal with 69% of correct discriminations, and higher SNR values would imply an increased probability that the waveform contains a signal.²³ Accordingly, SNR values were used for the separation of eyes to those with and without detectable mfERG responses, with a threshold of 1.4 as a cutoff for a detectable signal with a discriminability of >95%.²³ Two groups were formed based on the SNR data: one with detectable retinal function (DRF, $n = 15$; median age: 34 years; range: 15 to 63 years) and one with no central retinal function (NCRF, $n = 14$; median age: 32.5 years; range: 14 to 47 years).

FIGURE 2. Schematic overlay of the ETDRS subfields used in the OCT analyses and the mfERG hexagons.

Amplitudes and peak times of the P1 components of first-order kernels were measured. For the mfERGs the patient data were compared with the normal age-matched control group of our electrophysiological laboratory ($n = 50$; median age: 31.5 years; range: 23 to 42 years) using mean $\pm$ 2SD values as the criterion for abnormality. Pupils were dilated with cyclopentolate 0.5%; topical anesthesia was one drop of 0.45% oxybuprocaine. Each patient was optimally refracted before testing and then corrected for the viewing distance of 32 cm from the subject's eyes.

Best-corrected VA was recorded in Snellen equivalents and then transformed to logMAR (logarithm of the minimal angle of resolution). The thickness values of the intraretinal layers and the macula and logMAR visual acuities were compared between the three groups using mixed-model ANOVA followed by Newman-Keuls post hoc test. Linear correlation analysis was performed and Pearson correlation coefficients were used to assess the correlation between logMAR visual acuities, disease duration, and the thickness of the measured intraretinal layers. Statistical analyses were performed using commercial statistical analytics software (SPSS 15.0; SPSS Inc., Chicago, IL; and Statistica 8.0 Software; Statsoft Inc., Tulsa, OK). Because of the number ($n = 14$) of comparisons, Bonferroni adjustment was performed for the level of statistical significance, which was set at $P < 0.0036$.

RESULTS

Visual Function

LogMAR VA was significantly worse in the NCRF group compared with that in the DRF group and controls (1.00 ± 0.00 , 0.84 ± 0.26 , and 0.42 ± 0.22 for the Control, DRF, and NCRF groups, respectively). There was no significant age difference between the groups. A clinical example from all groups is shown in Figure 3, with corresponding structural and functional results.

Functional Alterations

Multifocal ERGs were reduced or nondetectable in all patients, with peripheral responses being more affected than central ones. There was no detectable mfERG in the NCRF group in any of the rings, whereas responses to rings 3 to 5 were undetectable in the DRF group. The response densities (RD), P1 amplitudes, P1 peak times, and SNR distributions among the groups are shown in Table 1. Thirteen of 15 eyes (88%) in the DRF group had detectable mfERG components only in response to the central foveal hexagon; two eyes (12%) showed

FIGURE 3. Clinical example of a healthy control, an eye from the DRF, and an eye from the NCRF group with respective structural and functional data. *First row:* horizontal cross-sectional OCT scans of the macula. *Second row:* corresponding macular thickness map. *Third row:* ring presentation (summarized values of traces according to eccentricity). *Fourth row:* trace arrays (topographic map of electric activity) of the mfERG recordings (note the differing calibration). Note that the thickness of the macula (of the eye) from the DRF group is decreased in the pericentral and peripheral region compared with the healthy eye. Also, macular thickness (of the eye) from the NCRF group in the central and pericentral area is decreased compared with (the eye from) the DRF group. The ring presentation of the DRF eye indicates an average peak of <10 nV/deg² in all eccentricities except ring 1 (central hexagon), whereas there is no visible peak in any eccentricities in the NCRF eye. Note also that there is a remarkably diminished central peak surrounded by some (very) low peaks of unusual shape (or delayed peaks) in the trace arrays of the DRF group, but there is no detectable central peak in the NCRF eye (most traces simply reflect background “noise”).

TABLE 1. Multifocal ERG Characteristics of the Patient Groups and the Age-Matched Normal Control Group

Characteristic	Normal (<i>n</i> = 50)	DRF (<i>n</i> = 15)	NCRF (<i>n</i> = 14)
RD, nV/deg ²			
Ring 1	129.00 (24.02)	26.60 (9.60)	ND
Ring 2	84.60 (12.05)	8.82 (3.32)	ND
Ring 3	59.30 (9.90)	ND	ND
P1 amplitude, μ V			
Ring 1	2.00 (0.38)	0.41 (0.25)	ND
Ring 2	1.85 (0.27)	0.20 (0.07)	ND
Ring 3	1.84 (0.33)	ND	ND
P1 peak time, ms			
Ring 1	38.07 (3.30)	39.60 (4.30)	ND
Ring 2	35.30 (2.47)	39.56 (5.70)	ND
Ring 3	34.60 (1.37)	ND	ND
SNR			
Ring 1	3.75 (1.63)	1.91 (0.38)	0.94 (0.20)
Ring 2	6.20 (1.80)	1.17 (0.26)	0.97 (0.23)
Ring 3	5.56 (1.53)	1.03 (0.12)	0.71 (0.15)

Results are expressed as means ($\pm$ SD). ND, not detectable.

additional detectable mfERG in ring 2. Both the mean RDs and mean P1 values in the DRF group in ring 1 were reduced to 20% of the normal mean. Mean peak time of P1 was within normal timing in the central hexagon.

Structural Changes in the Central Region

Total retinal thickness and ONL thickness in the central region did not significantly differ between the DRF group and controls. The total thickness of the retina and the ONL was significantly less in the NCRF eyes compared with that in both DRF eyes and controls (see Table 2 and Fig. 4A).

Structural Changes in the Pericentral Region

The ONL was significantly thinner in the pericentral region in both RP groups compared with that in controls, with no difference between patient groups. The GCL+IPL and INL+OPL did not differ significantly between the DRF and control eyes, whereas all these complexes were significantly thinner in the NCRF group compared with those in the DRF group. The RNFL did not differ between groups. The thickness

of the retina was significantly less in both patient groups compared with that in control eyes. Furthermore, NCRF eyes had significantly thinner macula in this region compared with that in DRF eyes (see Table 2 and Fig. 4B).

Structural Changes in the Peripheral Region

The ONL along with the GCL+IPL and the total retina was significantly thinner in the peripheral region in both RP groups compared with those in controls, but did not differ between patient groups. The INL+OPL did not show any significant difference in any of the comparisons. As opposed to the pericentral region, the RNFL in the peripheral region was significantly thicker in both patient groups compared with that in controls, but did not differ significantly between the two patient groups (see Table 2 and Fig. 4C).

The ONL and the total retina in the central region showed a significant linear correlation with logMAR VA ($r = -0.56$ and $r = -0.59$, $P = 0.003$ and $P = 0.002$, respectively) in both patient groups, such that better VA was associated with greater preservation of structure. There was no correlation between

TABLE 2. Regional Thickness of the Intraretinal Layers and the Total Retina

Variable	Thickness (mm), Mean (SD)			<i>P</i> Values		
	Control	DRF	NCRF	DRF vs. Control	NCRF vs. Control	DRF vs. NCRF
Central Region						
ONL	112 (7)	111 (16)	83 (12)	0.946	<0.001*	<0.001*
Total retina	229 (13)	245 (26)	186 (25)	0.036	<0.001*	<0.001*
Pericentral Region						
RNFL	25 (3)	28 (4)	22 (6)	0.050	0.233	0.006
GCL+IPL	93 (8)	99 (14)	77 (8)	0.041	0.001*	<0.001*
INL+OPL	75 (6)	78 (5)	66 (10)	0.275	0.003*	<0.001*
ONL	87 (5)	60 (11)	58 (16)	<0.001*	<0.001*	0.371
Total retina	317 (12)	305 (20)	262 (19)	0.205	<0.001*	<0.001*
Peripheral Region						
RNFL	43 (2)	57 (9)	54 (14)	<0.001*	0.002*	0.396
GCL+IPL	67 (4)	50 (9)	49 (11)	<0.001*	<0.001*	0.522
INL+OPL	64 (4)	66 (4)	66 (6)	0.344	0.385	0.978
ONL	75 (3)	44 (10)	46 (14)	<0.001*	<0.001*	0.667
Total retina	285 (7)	256 (22)	255 (20)	<0.001*	<0.001*	0.640

For statistical analyses, see the text and Figure 2.

* *P* values are considered statistically significant.

FIGURE 4. Retinal layer thickness results (A) in the central, (B) in the pericentral, and (C) in the peripheral regions. The black columns are for the Control group, the gray columns are for the DRF (decreased retinal function on mfERG) group, and the white columns are for the NCRF (no central retinal function on mfERG) group. The vertical bars denote the SD of the mean. *Statistically significant difference.

disease duration and any thickness parameters in either group (data not shown).

DISCUSSION

The present study correlates retinal structure and function in patients with RP using multifocal electroretinography and optical coherence tomography.

The limited histopathological literature on RP describes photoreceptor damage,^{2,24} with the earliest histologic change in all forms of RP being shortening of the rod outer segments (ROS), in keeping with diminished ROS renewal.² A significant reduction of cells occurs in all layers, principally the photoreceptors.^{2,24}

The in vivo evaluation of pathologic changes was difficult before recent advances in imaging technology.²⁵ OCT enables the in vivo assessment of retinal structure, with an axial resolution close to that of histology.²⁶ To date, various computer-aided procedures have been introduced to provide OCT-based quantitative information about the intraretinal structures.^{11,27,28} In this study a custom-built software that facilitates the segmentation of the various cellular layers of the retina was used to quantify pathologic changes of the macula and intraretinal structures. It has been previously shown that the OCTRIMA software, despite using information derived from time-domain OCT technology, enables highly reproducible measurements that are even comparable with spectral domain (SD)-OCT technology.^{12,14,29} In addition, it has been reported that patients with RP may develop cystoid macular edema or epiretinal membranes during the natural course of the disease, which makes reliable segmentation of the intraretinal layers impossible due to the disruptive structural changes of the retina.¹¹ Therefore, eyes with no evidence of cystoid macular edema were retrospectively selected for the study to provide reliable segmentation of the intraretinal layers.

The mfERG in RP can show well-preserved central retinal function,³⁰ but with disease progression the involvement of more central photoreceptors gives a consecutive decrease (or loss) of mfERG responses to the central mfERG hexagons; mfERGs depend on the integrity of cone photoreceptors, even though it is only the N1 component that has a significant cone photoreceptor contribution;³¹ much of the mfERG signal arises in the cone bipolar cells. In contrast to the markedly reduced response density and P1 amplitudes, the peak time of P1 to the central 5° is not delayed in RP patients with good central visual acuity, even if the cone-mediated full-field ERG is delayed.³⁰ This phenomenon can be explained by the size of the mfERG array that covers less than one quarter of the total cones stimulated in the full-field ERG and thus full-field ERG is dominated more by the periphery compared with the mfERG response.³⁰ The present results are in keeping with those data. Because the thickness of the ONL was preserved only in the central ETDRS region of those patients with detectable mfERG and was significantly thinner in those with undetectable mfERG responses, the current OCT results are in accordance with the electrophysiology. It is worthy of note, in relation to the visual acuity correlation, that the central foveal hexagon of the mfERG stimulus stimulates an area that corresponds to the central ETDRS area. Where no mfERG activity was detected (i.e., in the more peripheral regions), the ONL was significantly thinner in both patient groups compared with controls and showed a similar degree of photoreceptor loss.

Our results are in accordance with the photoreceptor thinning previously observed in the OCTs of patients with RP. Hood et al.⁵ reported thinning of the photoreceptor and RPE complex, and thinning of the macular photoreceptor outer segment and RPE complex has been reported with high-reso-

lution OCT.^{5,7} The findings are consistent with the histologic demonstration of shortening of ROS in RP.²

Although OCT demonstration of thinning of the photoreceptors is a consistent observation,^{4–7} studies describing changes in the INL and OPL are less conclusive.^{4,6,8–10} In the present study, there was no detectable change in the thickness of the middle layers of the peripheral macula (i.e., the INL and OPL), which were considered as a middle retinal complex (i.e., INL+OPL) for the segmentation analysis to obtain more precise measurements.¹⁴ Secondary degeneration affecting inner retinal cells as a consequence of photoreceptor degeneration could result in simultaneous reactive hyperplasia of the Müller glial cells that might counterbalance the putative loss of INL neurons.² However, the INL+OPL complex was significantly thinner in the pericentral region in the NCRF group, perhaps due to a different degree of the involuntary reaction of the INL and hypertrophic glial response. Previous histopathologic and OCT studies have shown no significant changes in the INL and OPL layers. Santos et al.²⁴ analyzed the sectioned maculae of 21 postmortem eyes with RP and found that a major part of the cells of the INL remained intact even in eyes with severe RP. Hood et al.⁵ found the thickness of the INL close to normal on spectral-domain OCT images measured with a manual segmentation procedure aided by a computer program.

The ganglion cell and inner plexiform layer complex in the pericentral region of those patients with detectable mfERG was preserved in the present study and thinning was observed only in the peripheral region. In contrast, the GCL+IPL complex was thinner in both peripheral and pericentral regions of those with undetectable mfERG responses. This may reflect more prominent photoreceptor damage and transneuronal degeneration in patients with undetectable mfERG responses giving a more pronounced thinning of the inner retinal layers, among them the ganglion cells. Since cone density is higher in the more central regions, photoreceptor loss and consecutive transneuronal degeneration may be less evident in the central and pericentral macula in less advanced stages of the disease than it is in the peripheral macula. Accordingly, the ganglion cells of the pericentral region are still seemingly preserved in eyes in which the underlying photoreceptors have already been reduced due to RP. However, the results of the NCRF group suggest that the ganglion cells in the pericentral region also degenerate with disease progression. The results of post-mortem morphometric studies, showing a significant reduction in the number of ganglion cells in eyes with moderate and severe RP, are consistent with the present *in vivo* results showing the thinning of ganglion cell layer in advanced RP.^{2,24} In contrast, Aleman et al.⁴ observed the thickening of the inner retina in association with the loss of ONL, whereas Hood et al.⁵ found the thickness of the GCL+IPL close to normal using images further processed with custom algorithms.

A pronounced thickening was observed for the RNFL in the peripheral region of both groups, which could be due to an early phase of epiretinal membrane formation. Although it was not among the aims of the present study to quantify this process, peripheral ERMs not involving the fovea (according to the exclusion criteria) were present in 41% and 42% in the DRF and NCRF groups, respectively. Based on histopathology, these membranes are formed by proliferation of fibrous astrocytes on the surface of the optic nerve head that progress to more peripheral areas.² For this reason, it is not surprising that the retina was thicker in the region closer to the optic disc, whereas in the pericentral region there was only a slight thinning detectable in the NCRF group, most probably due to the loss of the ganglion cells and their corresponding axons. Previous studies using OCT for the measurements of the peripapillary RNFL thickness showed conflicting results, reporting

both thinning and thickening of the retinal nerve fiber layer in the eyes of patients with RP.^{5,8–10}

The presumed trend cited earlier of gradual degeneration involving the ganglion cells due to prior photoreceptor loss is also supported by the total retinal thickness results. Specifically, in those eyes with detectable mfERG response, the thickness of the ONL in the central region representing the total retina is preserved. Moreover, the thickness of the retina in the pericentral region is significantly thinner than that in controls but at the same time thicker than that in those eyes with undetectable mfERG response, whereas the two RP groups have the same reduced thickness in the peripheral macula. A schematic drawing of the presumed trend of neurodegeneration occurring in RP based on the above-cited results can be seen in Figure 5.

Visual acuity in the present study was significantly worse in the NCRF group compared with that in both the DRF group and controls. The logMAR value showed a good correlation with the thickness of the ONL and the macula in the central region. Witkin et al.⁷ reported no correlation between central foveal thickness and logMAR VA; however, the correlation between macular photoreceptor outer segment–RPE thickness and logMAR VA was strong.

The Moorfields group has previously shown a correlation between the spatial extent of mfERG preservation and the radius of the paracentral ring of hyperautofluorescence in RP patients with good visual acuity. There was also spatial concordance between sensitivity loss measured by high spatial resolution fine matrix mapping and the size of the high-density hyperautofluorescent ring.^{19,32} Lima et al.³³ and Wakabayashi et al.³⁴ have both observed inner/outer photoreceptor segment junction disruption across the hyperautofluorescent ring, whereas outside the ring the inner/outer photoreceptor segment junction and the ONL appeared to be absent. Those data, and the correlation between structure and function found in the present study, point toward the need for a multimodal approach, to better understand the pathologic processes in RP.

There are some potential shortcomings of our study. On mfERG ring analysis, the “ring 1” central response is often the noisiest because it relates to a single stimulus hexagon and reliable amplitude measurement may be difficult, which could have biased the distinction between the two groups; we believe the use of SNR provides a relatively objective way to deal with this problem. Spatial correspondence between mfERG stimulus and OCT scans has been one of the major issues in all studies comparing structure and function.^{18,19,35} In our study, we used a 61 hexagonal-element pattern array that covered a 30° radius of the central visual field. The diameter of the first three mfERGs (only 19 hexagons) span 15° radius. Therefore, the OCT scan length (~20°) presumably covers approximately the diameter of these first three concentric mfERG rings. Consequently, the foveal and parafoveal retina are properly matched. A partial mismatch is present in the parafoveal region since the OCT scan slightly extends to ring 4. Thus, we note that the matching between the mfERG stimulus and the OCT scans needs to be improved in future studies by developing a proper geometrical model in a simulation study.

Regarding OCT technology, first, the ONL contains the inner segments of the photoreceptors that are interconnected with the outer segments of the photoreceptors and pigment epithelial cells. As previously mentioned, ultrahigh resolution and spectral-domain technologies facilitate a more precise delineation of the RPE and inner segment–outer segment junction of the macular photoreceptors,^{5,7} although the valuable retrospective database reaching back to 2006 used for the study made it impossible to include Fourier domain–OCT analyses. Although OCTRIMA is able to extract the RPE layer in time-domain OCT, there is much variability in the segmenta-

FIGURE 5. Schematic drawing of the observed pathologic changes in the macula of patients with RP. *Top:* normal structure of a healthy macula. *Middle:* schematic drawing of a macula in the DRF (decreased retinal function on mfERG) group. *Bottom:* image of a macula in the NCRF (no central retinal function on mfERG) group. Blue denotes thinning, whereas red is for thickening. Note that RNFL thickening occurs early in the peripheral region, whereas the pericentral ganglion cell layer (GCL+IPL) is affected only in the NCRF group despite the thinning of the pericentral ONL already in the DRF group.

tion of the RPE outer boundary due to the lower resolution of deeper structures extracted by the OCT device.¹⁴ Therefore, those measurements were not included. Although the INL and OPL layers could be better resolved by spectral-domain OCT technology, TD-OCT and SD-OCT have been shown to give comparable results when OCTRIMA is used.¹² Also, the results may be influenced by a combination of the quality of the scans (i.e., distinctiveness of the layers) and the segmentation algorithm performance. For example, a systematic deviation in the RNFL/GCL border could yield patients with thinner GCL+IPL, but thicker RNFL layers, although we believe the scan quality control in OCTRIMA along with previously demonstrated high reproducibility of OCTRIMA makes this error relatively unlikely.^{14,29} Future studies will benefit from higher resolution imaging; an increase in the size of the patient population studied will also be of importance along with longitudinal data.

Currently, there is no effective treatment for RP. The use of retinal implants is a possible avenue for the restoration of vision to the blind. However, the efficacy of such implants depends on the cells of the inner nuclear layer and the ganglion cell layer being functionally intact.³⁶ The quantitative structural data provided by OCT image segmentation could be a valuable tool in effective selection of patients, where the integrity of the ganglion cell layer is a prerequisite for potential use of retinal prostheses and could act as a useful outcome measure in therapeutic interventions.

Acknowledgments

The authors thank Balázs Varsányi for his valuable help with mfERG analyses and Balázs Lesch and Idikó Weiszer for their technical assistance and support.

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