Associations Between Outer Retinal Structures and Focal Macular Electoretinograms in Patients With Retinitis Pigmentosa

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Purpose. Our earlier study showed that the width of the intact ellipsoid zone (EZ) of the photoreceptors was significantly but weakly correlated with the amplitudes of the focal macular ERGs (FMERGs). The aim of this study was to determine a microstructure of the photoreceptors in the spectral-domain optical coherence tomographic (SD-OCT) images that was more strongly correlated with the FMERG parameters in eyes with retinitis pigmentosa (RP).

Methods. This was a retrospective, observational study. The medical records of 65 patients with RP were reviewed. FMERGs were elicited by a 15-degree stimulus spot. The width of the EZ and the outer segment (OS) area surrounded by EZ and retinal pigment epithelium in the SD-OCT images within 15 degrees of the fovea were evaluated. Spearman correlation tests and multiple stepwise regression analyses were performed.

Results. There was a strong correlation between the amplitudes of FMERGs and the EZ width ($r = 0.68$ for a-wave amplitude; $r = 0.64$ for b-wave amplitude), and also between the amplitudes of the FMERGs and the OS area ($r = 0.69$ for a-wave amplitude; $r = 0.67$ for b-wave amplitude). However, some patients had long EZ widths but had severely reduced FMERGs. Multiple stepwise regression analyses showed that the OS area was the only significant independent predictor of the amplitudes of FMERGs ($P < 0.001$).

Conclusions. The OS area might be a better morphological structure to use to predict the physiological function of the macula.

Keywords: retinitis pigmentosa, spectral domain optical coherence tomography, focal macular electoretinogram, photoreceptor
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than with the width of the EZ in retinas reattached by surgery for fovea off rhegmatogenous retinal detachment.1,3

The aim of this study was to determine an anatomical structure of the photoreceptors in the OCT images that was more strongly correlated with the FMERG parameters than the EZ width. To accomplish this, we measured the OS thickness as the distance between the EZ and the RPE as previous studies,1,19,20 and the OS area as the area bordered by the EZ superiorly, the RPE inferiorly, and was 15 degrees wide and centered on the fovea. We shall show that the OS area was a better predictor of the physiological condition of the macula especially in the early stages of RP.

METHODS

Subjects

We reviewed the medical records of patients with RP who were examined in the Nagoya University Hospital from March 2013 to October 2016. An oral informed consent was obtained from the patients after an explanation of the procedures and purpose of the examinations. The procedures used in this study conformed to the tenets of the Declaration of Helsinki, and Institutional Review Board/Ethics Committee approval was obtained (approval no. 2016-0538).

All of the patients had comprehensive ocular examinations including measurements of the best-corrected visual acuity (BCVA), fundus examinations by indirect ophthalmoscopy, fundus photography, slit-lamp biomicroscopy, visual field tests with Goldmann perimetry, and full-field ERGs. The diagnosis of RP was based on the ocular history, concentric contraction of the visual field, and pathognomonic features of the fundus (e.g., pigmentary changes and attenuation of the blood vessels). We excluded patients whose scotopic b-wave amplitudes of the full-field ERGs elicited by a bright flash (PE-3000; TOMEY, Nagoya, Japan) was >100 μV. We identified 99 patients who met the inclusion criteria and had FMERGs recorded. Generally, FMERGs were recorded from patients who had relatively good visual acuity (e.g., >20/25), because we found that the FMERGs became nonrecordable in the advanced stage of RP.10 We excluded 18 patients whose b-wave amplitudes of FMERGs were <0.5 μV because of the difficulty in measuring FMERG parameters. We also excluded 16 patients with cystoid macular edema identified in the spectral-domain OCT (SD-OCT) images because these changes prevented us from quantifying the structure of outer retina accurately. If both eyes met the inclusion criteria, then the data from only the right eye were used for the statistical analyses. Based on these criteria, 65 eyes of 65 patients with RP were analyzed.

The FMERG and SD-OCT data of 43 normal fellow eyes of 43 subjects who had unilateral retinal diseases were analyzed in the same way, and the findings of fellow eyes were used as normal control data for comparison to the data of the RP patients.

Focal Macular ERGs

The procedures used for recording FMERGs (ER-80; Kowa, Nagoya, Japan) have been described in detail.11 Briefly, the pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. A Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Laboratories, Iowa City, IA, USA) was used to record FMERGs. The diameter of the stimulus spot was 15 degrees, the luminance of the stimulus was 30 cd/m², the background luminance was 3 cd/2 m², and the stimulus duration was 100 ms. The position of the stimulus spot on the fundus was monitored during the recording with a modified infrared fundus camera.

The responses were recorded with digitally bandpass filters set at 5 to 500 Hz for the a- and b-waves, and 500 responses were averaged. The frequency of stimulation was 5 Hz (Neupack S1 MEB-9400; Nihon Kohden, Tokyo, Japan). The waveforms with frequencies >70 Hz were extracted from the raw FMERGs by Fast Fourier Transform (FFT; Fig. 1A). This process allowed us to isolate and measure the amplitudes and implicit times of a- and b-waves by filtering out the oscillatory potentials (OPs) and the high frequency noise.

The amplitude of a-wave was measured from the baseline to the first negative trough and that of b-wave was measured from the trough of a-wave to the next large positive peak. The implicit times of a- and b-waves were measured from the stimulus onset to the trough of a-wave and the peak of b-wave. Stimulus markers are shown beneath each waveform. (B) Representative horizontal cross-sectional SD-OCT image of a patient with RP within a 15-degree foveal area. We defined the lateral borders of the EZ as the locations where the upper surface of the EZ (shown as red line) meets the upper surface of the RPE (shown as blue line). The EZ width was measured between the edge of the EZ (left tip of arrow shown as broken line) or the borders of the 15-degree area (right tip of arrow shown as broken line). The OS area (in yellow) is defined as the area surrounded by the lower surface of the EZ (shown as green line) and upper surface of the RPE (shown as blue line), and 15 degrees wide.

SD-OCT Measurements

All eyes had cross-sectional OCT images that were obtained by radial scanning of 30 degrees diameter with the Spectralis SD-OCT instrument (Heidelberg Engineering, Heidelberg, Germany). All images were the average of 100 SD-OCT scans using the eye tracking system. Only the horizontal and vertical scans centered on the fovea were evaluated. To compare the FMERGs and SD-OCT findings, the SD-OCT images centered on the fovea and 15 degrees in diameter were used for the analyses.
Width of EZ

One masked examiner (AK) measured the EZ width in the SD-OCT images manually within 15 degrees in diameter of the fovea using the built-in caliper function in the Spectralis OCT. We set the borders of the EZ as the location where the EZ band met the upper surface of the RPE because of the ease of identifying this, although several previous studies determined it to be the midpoint between the upper and lower surface of the RPE. If the EZ width was longer than 15 degrees, the lateral border of the EZ was set as the edge of analyzed area of 15 degrees. Then, we defined the EZ width as the lateral distance between the borders as reported (Fig. 1B). The average value of the EZ width in the horizontal and vertical scan images was used for the statistical analyses. We considered these two sections were reliable indicators for the actual area as reported.

OS Area

The OS area was measured from the lower surface of the EZ and the upper surface of the RPE within 15 degrees diameter of the fovea in the SD-OCT images (Fig. 1B). The area bordered by the EZ superiorly and the RPE inferiorly, and was 15 degrees wide was designated as the OS area. The average of the OS area in the horizontal and vertical SD-OCT images was used for the statistical analyses.

One masked examiner (AK) marked the areas of interest and determined the size by the number of pixels using the ImageJ software (version 1.48; http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The number of pixels was converted to metric units (μm) using the resolution (μm/pixel) fixed in the software of each OCT image for both vertical and horizontal scans.

Statistical Analyses

Mann-Whitney’s U tests were used to determine the significance of any differences in the FMERG and SD-OCT parameters between RP and control groups. Spearman correlation tests were used to determine the significance of the correlations between the FMERG and SD-OCT parameters. Multiple stepwise regression analyses were used to evaluate which independent variable including the BCVA, age, the EZ width, and the OS area can be a reliable determinant of the FMERG amplitudes. A P < 0.05 was taken to be statistically significant.

RESULTS

Comparisons of Clinical Characteristics and FMERG and SD-OCT Parameters of RP Eyes to Control Eyes

The inheritance pattern was autosomal dominant in 10 (15%) patients, autosomal recessive in 10 (15%), and sporadic in 45 (70%). We classified patients who had no familial histories of RP as sporadic. None of the patients had X-linked RP. The demographic and clinical characteristics of the 65 patients (24 men and 41 women) with RP and 43 control eyes are shown in Table 1. The mean BCVA was not significantly different between the two groups because only RP patients with BCVA better than 20/25 were studied. However, the values of the other parameters were significantly different between RP and controls (Mann-Whitney’s U test).

Representative Cases

The FMERGs, horizontally scanned SD-OCT images, and fundus photographs of three eyes with RP (cases 1–3) and one control eye are shown in Figure 2. The fundus photographs of the RP patients show the characteristic features of RP although there were differences in the degree of pigmentation.

Case 1 was a 34-year-old woman with RP. She was a representative case with a long EZ and large FMERG amplitudes. The amplitudes of a- and b-waves were 1.23 and 2.07 μV, respectively, both of which were only slightly smaller than the averages of those in the normal subjects. The implicit times of a- and b-waves were 23.4 and 42.6 ms, respectively, and the implicit time of a-wave was longer than the average of the normal subjects. The EZ was visible with a width of 496 μm in the 15-degree image, and the CIZ was also visible beneath the EZ. The OS area was 0.110 mm².

Case 2 was a 64-year-old man. He was a representative case with a long EZ with small FMERG amplitudes. The amplitudes of a- and b-waves were 0.66 and 0.84 μV, respectively, and the amplitude of the b-wave was lower than the average of that in RP patients. The implicit times of the a- and b-waves were 24.5 and 41.7 ms, respectively, and the implicit time of the a-wave was longer than the average of that in the normal subjects. Although EZ was detected over the 15 degrees of the SD-OCT image (width, 4780 μm), the CIZ was barely detectable. The OS area was 0.092 mm², which was smaller than that of case 1. Comparisons of cases 1 and 2 showed that the amplitudes of the FMERG parameters were smaller in the patient with smaller OS area.

Case 3 was a 24-year-old man. He was a representative case with a short EZ and reduced FMERG amplitudes. The amplitudes of the a- and b-waves were 0.21 and 0.76 μV,
respectively, and both were markedly smaller than the averages of those in the normal subjects. The implicit times of the a- and b-waves were 24.5 and 45.0 ms, respectively, both of which were longer than the averages of those in the normal subjects. The EZ was visible for approximately 5 degrees in the center, which was much shorter than those in cases 1 and 2. The EZ width was 1760 μm, and the CIZ was just barely visible in the central area. The OS area was 0.039 mm².

Case 4 was a 40-year-old man who is a representative case of RP. The EZ is preserved but the CIZ is not visible. Fundus color photograph shows a narrowing of the vessels. Case 3 was a 24-year-old man with RP and is a representative case with short EZ and small FMERG amplitudes. The EZ is short and the CIZ is barely visible. Fundus color photograph shows retinal degeneration. Case 2 was a 64-year-old man with RP and is a representative case with long EZ and small FMERG amplitudes. The EZ is preserved but the CIZ is barely visible. Fundus color photograph shows retinal degeneration. Case 1 was a 34-year-old woman with RP and is a representative case with preserved long EZ with large FMERG amplitudes. The EZ and CIZ are fully detectable in the SD-OCT images.

The amplitudes of the a- and b-waves of the 65 RP patients were 22.6 and 42.0 ms, respectively. The CIZ was visible throughout the OCT image. The EZ width was 4816 μm, and the OS area was 0.150 mm².

**Correlations Between the EZ Width and the FMERG Parameters in Patients With RP**

We examined whether the FMERG parameters were significantly correlated with the EZ width in eyes with RP. The implicit times of a- and b-waves of the 65 RP patients are plotted against the EZ width in Figures 3A and 3B, respectively. Significant correlations were found between the EZ width and the implicit times of a- and b-waves (a-wave, r = -0.34, P = 0.005; b-wave, r = -0.64, P < 0.001, Spearman correlation test).

The amplitudes of the a- and b-waves of the 65 RP patients are plotted against the EZ width in Figures 3C and 3D, respectively. Significant and relatively strong correlations were found between the EZ width and the amplitudes of a- and b-waves (a-wave, r = 0.68, P < 0.001; b-wave, r = 0.64, P < 0.001, Spearman correlation test).

**Correlation Between the OS Area and FMERG Parameters in Patients With RP**

We also determined whether the FMERG parameters were significantly correlated with the OS area in eyes with RP. The implicit times of a- and b-waves of 65 RP patients are plotted against the OS area in Figures 4A and 4B, respectively. Significant correlations were found between the OS area and the implicit times of a- and b-waves (a-wave, r = -0.40, P = 0.001; b-wave, r = -0.65, P < 0.001, Spearman correlation test). The amplitudes of a- and b-waves of 65 RP patients are plotted against the OS area in Figures 4C and 4D, respectively. Significant correlations were found between the OS area and the amplitudes of a- and b-waves (a-wave, r = 0.69, P < 0.001; b-wave, r = 0.67, P < 0.001, Spearman correlation test).

We next performed multiple stepwise regression analyses to determine which parameter including the OS area, the EZ width, the BCVA, and age were significant independent factors for predicting the FMERG amplitudes. The results showed that only the OS area was a significant predictor of the amplitudes of a- and b-waves of FMERGs (Table 2).
Correlations Between the OS Area and the FMERG Parameters in RP Patients With Preserved EZ Width

Although the EZ width was significantly correlated with the amplitudes of a- and b-waves, there were several cases with long EZ but with small FMERG amplitudes as has been reported.\(^{15,16}\) We noted that the OS area of some cases with long EZ and small b-wave amplitudes were relatively small (cases 2, 25, and 63 in Fig. 5). Thus, we performed subgroup analysis of patients who had longer EZ widths than the average of the 65 RP cases (3701 µm) and re-evaluated the correlations between the FMERG parameters. Our results showed that the OS area was significantly correlated with the EZ width when the average of the 65 RP cases (3701 µm) and re-evaluated the correlations between the FMERG parameters. Our results showed that the OS area was significantly correlated with the EZ width ($r = 0.50, P = 0.003$, Spearman correlation test), but the EZ width was not significantly correlated with the FMERG b-wave amplitude ($r = 0.29, P = 0.09$, Spearman correlation test; Fig. 5).

DISCUSSION

Earlier, we evaluated the relationship of the FMERG parameters and the OCT findings in RP patients but two problems were encountered.\(^{16}\) First, we could not evaluate the relationship between the implicit times of a- and b-waves and the OCT parameters because we included many patients whose amplitudes of the FMERGs were so reduced that the implicit times could not be measured accurately. The second problem was that we could not understand and interpret the findings in some RP patients who had severely reduced FMERG amplitudes although the EZ was relatively well-preserved. In addition, the presence of these patients might have lowered the correlations between the EZ widths and the FMERG amplitudes.

To try to resolve these difficulties, we analyzed a relatively larger sample of patients and studied only patients who had b-wave amplitudes of FMERGs more than 0.5 µV. In addition, the advancements of the imaging devices allowed us to obtain more accurate values of the microstructure of the photoreceptors. As a result, higher coefficients of correlations were found between the EZ width and the FMERG amplitudes. The differences in the inclusion criteria of the patients in the present study might have contributed to the relatively stronger correlations than those of the previous study. The fact that the FMERG amplitudes were significantly correlated with the EZ widths was not surprising because the number of cones that give rise to the electrical potentials are believed to decrease corresponding to the shortening of the EZ width. However, even in the present study, there was a large variation in the amplitudes of FMERGs, and some patients had severely reduced amplitudes although the EZ width was relatively well-preserved, the so-called “dysfunctional EZ”.\(^{16}\) Thus, we performed subgroup analysis of patients with preserved EZ, and the results showed no significant correlation between the amplitude of FMERG b-wave and the EZ width.

Our results also showed that there were significant prolongations of the implicit times of the FMERGs in RP patients. These findings are in agreement with the results of focal ERGs, mERGs, and full-field ERGs.\(^{27-34}\) The implicit times represent the speed of the transduction processes in the retina, and a prolongation of the implicit times would be related to abnormalities of the transduction process in the cones and their downstream pathway. The implicit times of a- and b-waves of the FMERGs were negatively correlated with the EZ width and the OS area. These results indicate that the transduction process of the residual retinal neurons become slower as the RP disease process advances. Thus, the implicit times can be another important indicator of functional changes in the macula area of patients with RP.

### Table 2. Results of Multiple Stepwise Regression Analysis for Independence of Factors Contributing to FMERG Amplitudes

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<th>Variable</th>
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<th>P Value</th>
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<td>of a-wave</td>
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<tr>
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<td>of b-wave</td>
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</tr>
<tr>
<td>FMERG amplitude</td>
<td>OS area</td>
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</tr>
<tr>
<td></td>
<td>of b-wave</td>
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<tr>
<td></td>
<td>Adjusted $R^2 = 0.45$</td>
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**FIGURE 5.** Correlations between the SD-OCT parameters and the amplitudes of b-waves in subgroup whose EZ width were longer than the average of the RP cases. The FMERG b-wave amplitude was not significantly correlated with the EZ width (A) but was with the OS area (B). The numbers written in red in (A) and (B) correspond to each other.
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This study has some limitations. First, RP refers to a diverse group of inherited retinal degenerative disorders caused by mutations in different photoreceptor-associated genes. However, the relationship between the gene mutations and the microstructures of eyes with RP was not analyzed because the causative gene mutations were determined in only a few patients. It has been reported that differences in the causative gene can affect the cone mosaics differently, and evaluating the relationship between the causative gene and FMERG parameters would be of interest.

Second, we did not analyze the mfERGs but the FMERGs. The mfERGs can assess the function of multiple retinal areas mathematically, and it is suitable to analyze retinal function of specific areas. However, the amplitudes of each response is small, but the focal ERGs are elicited directly from macular area while monitoring the location of the stimulus spot on the macula. The amplitudes of FMERGs are relatively large, and FMERGs are composed of the a-, b-waves and OPs. We aimed to evaluate the total residual macular function of RP patients, and the use of FMERGs seemed to be more appropriate for our aim. In addition, our laboratory has studied a wide range of retinal diseases using FMERGs, therefore, we adopted the FMERGs to evaluate macular function.

Third, we were not able to evaluate the CIZ because it was difficult to judge the presence of the CIZ in some patients. However, we assumed that the presence of CIZ was associated with better cone function in RP because the CIZ is part of the OSs of the photoreceptors.

In conclusion, we evaluated the residual macular function of the retina by FMERGs, and determined the association with the OCT parameters at a relatively early stage of RP. We found that the EZ width and the OS area were important factors that were significantly correlated with large FMERG amplitudes with short implicit times. Evaluations of the EZ width in RP is important, but the foveal cone density can be decreased in RP before visible changes are detected in the OCT images even while the visual acuity and foveal sensitivity are good. On the other hand, the FMERGs represent the mass of electrical potentials evoked from cones, and it might reflect the status of cone photoreceptors. The assessment of the OS area is probably associated with the functional status of the cones and could provide important information for determining the macular function in RP because only the OS area was found to be a significant predictor of the FMERG amplitudes.

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19. Hood DC, Lin CE, Lazow MA, Locke KG, Zhang X, Birch DG. Thickness of receptor and post-receptor retinal layers in patients with retinitis pigmentosa measured with frequency-


