Retinitis pigmentosa (RP) is a genetically heterogeneous group of inherited retinal disorders with degeneration of the rod and cone photoreceptors. The fundus of patients with RP shows pathognomonic alterations including changes in the retinal pigment epithelium (RPE), attenuation of the blood vessels, and peripheral bone-spicule pigmentation. The symptoms and signs of RP are impaired night vision, slow progressive peripheral-to-central visual field loss, and a reduction of the visual acuity. The residual visual function in RP is usually evaluated by tests of the visual fields by Goldmann kinetic perimetry, static visual field tests, and microperimetry.

Recent advances in optical coherence tomography (OCT) have enabled clinicians to assess the anatomical alterations of the microstructures of the retina in different types of retinal diseases, and many researchers have reported significant correlations between the visual functions and the integrity of the photoreceptor microstructures analyzed in the OCT images in eyes with RP. The integrity of the ellipsoid zone (EZ), previously referred to as the inner segment (IS)/outer segment (OS) border, in the ISs of the cones has received extensive attention in RP studies. This is because the width of the intact EZ (EZ width) was found to be significantly correlated with the diameter of the visual field and the amplitude of multifocal ERGs (mfERGs). Because of the ease and reliability in measuring the EZ width, the EZ width has been proposed as a possible anatomical measure of the retinal function in clinical trials of patients with RP.

Focal macular ERGs (FMERGs) are elicited by focal light stimuli, and they represent the neural activity of the neurons only in the macular area. The amplitudes and implicit times of the FMERGs have been reported to be significantly associated with the physiological condition of the macula in normal and in diseased eyes. We and another group have determined the correlations between the values of the FMERG parameters and the OCT parameters in eyes with RP. Unlike the relationship between visual field diameter and the EZ width, only a weak correlation was found between the amplitudes of the FMERG and the EZ width in two previous studies. Similar differences between the results of OCT and mfERGs in patients with a decrease in vision with relatively normal appearing retina have been reported. One of the reasons for the weak correlations between the EZ widths and the FMERG parameters was suggested to be that some RP patients who had long EZ widths also had severely reduced FMERGs, called “dysfunctional EZ.” The results of an earlier study suggested that the FMERG parameters were more strongly correlated with the width of the cone interdigitation zone (CIZ)
than with the width of the EZ in retinas reattached by surgery for fovea off rhegmatogenous retinal detachment.1,11

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.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., pigmented 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.16 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 patients. The amplitude of a-wave was measured from the baseline to the trough of a-wave and the peak of b-wave (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.

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.
considered these two sections were reliable indicators for scan images was used for the statistical analyses. We average value of the EZ width in the horizontal and vertical distance between the borders as reported (Fig. 1B). 22 The 15 degrees. Then, we defined the EZ width as the lateral border of the EZ was set as the edge of analyzed area of the RPE.4,6–9,21 If the EZ width was longer than 15 degrees, the it to be the midpoint between the upper and lower surface of the RPE.4,6–9,21 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).22 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.5

### 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.4,6–9,21 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).22 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.5

### 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).23 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 45 control eyes are shown in Table 1. The mean BCVA was not significantly different between the 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 4960 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,
The amplitudes of a- and b-waves were 24.5 and 45.0 ms, respectively, both of which were longer than the averages of those of the normal subjects. 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 EZ width was 1760 μm, which was much shorter than those in cases 1 and 2. The EZ is short and 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 and small FMERG amplitudes. 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 reduced FMERG amplitudes. The EZ is short and the CIZ is barely visible. Fundus color photograph shows retinal degeneration. Case 4 was a 40-year-old man who is a representative case of RP patients with short EZ and small FMERG amplitudes. CIZ is also visible beneath the EZ. Fundus color photograph shows retinal degeneration. The normal control was 40-year-old man. The EZ is preserved but the CIZ is not visible. Fundus color photograph shows a narrowing of the vessels.

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. 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 FMERG b-wave amplitude (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. 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.” 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.

An earlier study showed that the photoreceptor OSs were lost in the earliest stage of RP, followed by loss of the ISs, and finally loss of the photoreceptor nuclei in eyes with RP. To investigate the reason for the presence of cases with “dysfunctional EZ,” we focused on the OS area. Multiple stepwise regression analyses showed that only the OS area was a significant predictor for the FMERG amplitudes, and the OS area was correlated with the amplitude of b-waves even in the subgroup analysis including patients with “dysfunctional EZ.” Wen et al. reported similar results using mfERGs in 10 RP patients; the amplitudes of the mfERGs at each location were highly correlated with the thicknesses of the outer retinal layers in patients with RP, and they were not significantly correlated with the thicknesses of the inner nuclear layers and total retina. They found that the thicknesses from the RPE to the outer plexiform layer (REC-) and from the RPE to the EZ (OS+) were correlated with the amplitudes of the mfERGs. However, they did not conclude which is more strongly correlated with the mfERG amplitudes.

The EZ is part of the ISs of the cones, and the OS area includes the CIZ and other parts of the photoreceptor OSs. It has been shown that disturbances of the OS are accompanied by a reduction in the amplitudes of FMERGs. Thus, the “dysfunctional EZ” might indicate a loss of the OSs before disturbances of the ISs of the photoreceptors. In support of this, the results of several recent studies showed that the cone density in the images obtained by adaptive optics scanning laser ophthalmoscopy (AOSLO) in RP patients ranged from normal to severely reduced in spite of the presence of intact EZ in the OCT images. Further studies analyzing the relationships between the density of the cone mosaics in the AOSLO images and the FMERG parameters in RP patients are needed.

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, mfERGs, and full-field ERGs. 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.
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 amplitude 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 when 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.

Acknowledgments
The authors thank Duco Hamasaki, PhD (Bascom Palmer Eye Institute) for the discussions and editing the final version of the manuscript and Kazuki Nishida, MD (Department of Biostatistics, Nagoya University Graduate School of Medicine) for the discussions about statistical analyses.

Disclosure: T. Kominami, None; S. Ueno, None; A. Kominami, None; A. Nakasuali, None; S. Yasuda, None; C.-H. Piao, None; S. Okado, None; H. Terasaki, None

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