Correlation between Macular Volume and Focal Macular Electoretinogram in Patients with Retinitis Pigmentosa

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PURPOSE. To determine whether a significant correlation exists between the morphology of the macula measured by optical coherence tomography (OCT) and the amplitude of focal macular electoretinograms (fmERGs) in patients with retinitis pigmentosa (RP).

METHODS. fmERGs were recorded in 43 patients with RP and 43 age-similar normal subjects, with a 15° stimulus spot, 5.6 to 5.8 mm in diameter on the fundus. The sum of the volume of the neural retina in the central 6 mm (total macular volume) was measured with the OCT system. The length of the photoreceptor inner segment/outter segment junction (IS/OS line) in a 6-mm diameter macular area was also measured in the OCT images.

RESULTS. There was a weak correlation between the total macular volume and the fmERG amplitudes (correlation coefficient, 0.46 for the a-wave and 0.54 for the b-wave). The fmERG amplitudes in the patients with RP with IS/OS line longer than 2 mm were significantly larger than those in patients with RP with IS/OS line shorter than 2 mm, but the correlations between these two factors were weak. One major reason for the low correlations between the macular morphology and fmERGs was that there were some patients with RP who had normal macular volume and long IS/OS line, but had severely reduced focal macular ERGs.

CONCLUSIONS. Although the macular volume and length of the IS/OS line correlated weakly with the amplitude of the fmERGs, a preserved macular morphology does not necessarily guarantee normal-amplitude fmERGs in patients with RP. (Invest Ophthalmol Vis Sci. 2008;49:3551–3558) DOI:10.1167/iovs.08-1954

The inheritance pattern was autosomal dominant in 6 (14%) patients, autosomal recessive in 6 (14%), and sporadic in 31 (72%). None of the patients was found to have X-linked RP. The best corrected visual acuity ranged from 0.3 to 1.2, and the mean logarithm of the minimum angle of resolution (logMAR) was 0.052 units.

For controls, fmERGs and OCT were recorded from 43 age-similar normal subjects (14 males, 29 females; mean age, 42.7 years, range, 18–79 years). The mean ages of the RP group and normal controls were comparable (mean age = 45.8 ± 15.6 years and 47.4 ± 12.7 years, respectively). There was no statistically significant difference in the age distribution between the two groups. The patients who were 70 years or older were excluded (n = 4). In the normal control group, the eyes met these criteria, then the data from only the right eye were used for the analyses.

Disclosure: T. Sugita, None; M. Kondo, None; C.-H. Piao, None; Y. Ito, None; H. Terasaki, None

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16–67). None had known abnormalities of the visual system, and their visual acuity was ≥1.0 in all.

The research was conducted in accordance with the Institutional Guidelines of Nagoya University and conformed to the tenets of the World Medical Association’s Declaration of Helsinki. Informed consent was obtained from each of the patients after they were provided sufficient information on the procedures to be used.

**Focal Macular ERGs**

The stimulus and recording systems used to record fmERGs have been described in detail. Briefly, an infrared fundus camera equipped with a stimulus light, background illumination, and fixation target was used. The image from the camera was fed to a television monitor, and the examiner used the image on the monitor to maintain the stimulus on the macula. A stimulus spot size of 15° was selected because ocular biometry has shown that a 15° stimulus spot covers a retinal area of 5.5 to 5.8 mm, which is approximately the size of the OCT-determined macular diameter (6.0 mm). The background light subtended a visual angle of 45°, and additional background illumination outside the central 45° produced a homogeneous background for nearly the entire visual field. The luminances of the white stimulus light and background light were 29.46 and 2.89 cd/m², respectively. Although this luminance of background light was not strong enough to suppress all the rod activity, we have shown that the fmERGs elicited by this method are generated mainly by the cone system, and the responses elicited by spot stimuli of 5 to 15° are local responses.

A Burian-Allen bipolar contact lens electrode was used to record the fmERGs. This contact lens electrode system had low electrical noise and permitted a clear view of the fundus by the camera during the recordings. After the pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride, fmERGs were elicited by a flicker train consisting of a square waves presented at 5 Hz (100-ms on and 100-ms off). Then, a series of 512 responses were averaged in a single cycle by a signal processor. The time constant of the bioamplifier was set at 0.03 seconds with a 100-Hz high-cut filter to record the a- and b-waves.

The amplitude of the a-wave was measured from the baseline to the first negative trough, and the amplitude of b-wave was measured from the trough of the a-wave to the positive peak of the b-wave.

**OCT Measurements**

The morphology of the macula was evaluated by a high-resolution optical coherence tomograph (Stratus model 3000, software ver. 4.0.1; Carl Zeiss Meditec, AG, Oberkochen, Germany). After the patients’ pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine, the volume of the neural retina in the central 6 mm of the macula (total macular volume) was measured using six scans of 6 mm in a radial pattern intersecting at the fixation point.

It is known that the automatic fast macular thickness map (FMTM) protocol often fails to identify the outer borders of the neural retina, which can lead to recording of erroneous retinal thicknesses and volumes. Therefore, we used a program developed in our laboratory (Ishikawa K, et al. IOVS 2005;46:ARVO EAbstract 1550), by which the total macular volume was measured more precisely than that calculated by the conventional FMTM system. In this program, the user was able to set 20 cursors above and below a selected area manually. The inner cursors were set on the internal limiting membrane (ILM), and the outer cursors were set on the retinal pigment epithelium (RPE)-choriocapillaris hyperreflective complex borderline. Another set of cursors was set on the fovea of the OCT images. Then, each OCT radial scan was analyzed as a retinal map, and the total macular volume was calculated precisely by our software.

Past studies with the Stratus OCT and ultrahigh-resolution OCT demonstrated that there are two well-defined, parallel, highly reflective lines (HRLs) in the outer retinal layer. It has been shown that the inner HRL corresponds to the photoreceptor inner/outer segment junction or the IS/OS line, and the outer HRL corresponds to the retinal pigment epithelium and choriocapillaris complex. To assess the relationship between the morphologic changes in the photoreceptor layer and the amplitude of the fmERGs, we classified the IS/OS line in patients with RP into three types: type 1, distinct IS/OS line longer than the central 2 mm; type 2, distinct IS/OS line only within the central 2 mm; and type 3, absence of IS/OS line within the central 6 mm (Fig. 1). To perform this classification, we reviewed the six tomographic images of each eye on a gray scale with an alignment image protocol, because the IS/OS line is more clearly visible on gray-scale tomographic images. The classification was performed by TS in a masked manner.

**Statistical Analyses**

The significance of the differences between the patients with RP and normal control subjects was determined by nonparametric Mann-Whitney U tests. The correlations between the macular volume and the fmERG amplitudes were determined by the Spearman’s rank correlation. Differences in the amplitudes among the three groups (types 1, 2, and 3) based on the length of the IS/OS line were analyzed with the nonparametric Kruskal-Wallis test and Scheffe’s test, as the multiple comparison procedures. Differences and correlations were considered to be significant when \( P < 0.05 \).

**Results**

Representative OCT images and fmERGs recorded from one normal subject and three patients with RP are shown in Figure 2. The amplitudes of the fmERGs in case 1 were relatively well preserved, and the macular volume was within the normal range. The amplitudes of the fmERGs in case 2 were reduced, and the macular volume was close to the lower borderline of normal. The fmERGs in case 3 were nonrecordable, and the macular volume was severely reduced.

Box plots of the fmERG amplitudes (a- and b-waves) and total macular volume for 43 normal control subjects and 43 patients with RP are shown in Figure 3. As expected, both the amplitudes of the a- and b-waves of the fmERGs and the total

![Figure 1](image-url)
macular volume in patients with RP were significantly smaller than those of normal subjects ($P < 0.001$).

**Correlation between Amplitude of fmERG and Macular Volume**

Because changes in the macular morphology should lead to functional changes,52 we investigated whether there was a correlation between the amplitude of fmERGs and the total macular volume in our 43 patients with RP. The amplitudes of the a- and b-waves for 43 patients with RP are plotted against the total macular volume in Figures 4A and 4B, respectively. For both graphs, the gray area shows the 2.5 to 97.5 percentiles of normal control subjects.

A significant but weak correlation was found between the fmERG amplitude and total macular volume (a-wave, $\rho = 0.458$, $P < 0.01$; b-wave, $\rho = 0.540$, $P < 0.01$; Spearman’s rank correlation). One of the reasons for this relatively weak correlation between the fmERG amplitude and total macular volume was that there were four patients with RP who had normal macular volume but severely reduced fmERG (e.g., patients 4–7, Fig. 4). In contrast, there were no patients with RP who had normal a- and b-wave amplitudes with severely reduced macular volume. There were two patients with RP who had normal a-wave amplitude with reduced macular volume, but their macular volumes were still near the lower borderline of normal, and their b-wave amplitudes were lower than the normal range.

**Correlation between fmERG and Length of IS/OS Line**

We attempted to measure the thickness of each retinal layer (i.e., outer, middle, and inner retinal layers) separately, but found that it was very difficult to identify the border between these layers, especially in patients with relatively advanced stages of RP. The total macular volume is the sum of the volume of the neural retina in the central 6 mm of the retina and was used in the analyses. In addition, we used the length of the photoreceptor inner segment/outer segment junction

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**Figure 2.** OCT images and fmERGs recorded from a normal subject and three representative patients with RP.
(IS/OS line) as a measure of the structural integrity of the macular area.

The amplitudes of the fmERG for the three RP groups classified by the length of the IS/OS line are shown in the upper traces of Figure 5 (see also Fig. 1). The amplitudes of the fmERGs in type 1 patients with RP (distinct IS/OS line over the central 2 mm) were significantly larger than those in type 2 (distinct IS/OS line only in the central 2 mm) and type 1 (absent IS/OS line) patients with RP ($P < 0.05$). Nine (81%) of 11 patients with type 3 RP had nonrecordable fmERGs, whereas none with type 1 had nonrecordable fmERGs (Fig. 5, bottom plot). These findings suggest that the patients with RP with longer IS/OS lines had larger fmERG amplitudes.

However, we found that the correlation between the amplitude of the fmERGs and changes in the OCT image was weak, even when the integrity of the IS/OS line was used to separate the patients with RP into the three groups. The weak correlation was probably due to two factors: first, there was no statistically significant difference in the fmERG amplitude between types 2 and 3 ($P = 0.07$ for a-wave; $P = 0.20$ for b-wave); and second, there was a large variation in the amplitudes of the fmERGs in type 1 and some patients had severely reduced amplitudes (Fig. 5, bottom plot).

Patients with RP with Normal Macular Volume but Severely Reduced fmERGs

Finally, we wanted to investigate whether the IS/OS line was preserved in our four patients with normal macular volume and severely reduced fmERG amplitudes (Fig. 4). We expected that even though the total macular volume was within the normal range, these patients may have had a very short IS/OS line, which may be the reason for severely reduced mfERG. The gray-scale OCT images and the waveforms of fmERG in four patients with RP who had normal macular volume and severely reduced fmERG amplitude (patients 4–7) are shown.

![Figure 3](image3.png)

**Figure 3.** Box plots of the a- and b-waves of the fmERGs and total macular volume for normal controls and patients with RP. Line within the box indicates the median, the box the 25 and 75 percentiles, and the end of the error bars the 2.5 and 97.5 percentiles.

![Figure 4](image4.png)

**Figure 4.** Amplitudes of a- and b-waves plotted against total macular volume in 43 patients with RP. There is a weak but significant correlation between the fmERG amplitude and total macular volume. There were four patients with RP who had normal macular volume but severely reduced fmERG (patients 4–7). Shaded area: the 2.5 to 97.5 percentiles of total macular volume and mfERG amplitude in age-similar normal subjects.
in Figure 6. Against our expectations, the length of IS/OS line was relatively well-preserved (>4 mm) for these four patients, and was more than 5 mm for three patients (patients 4, 5, and 7). These results indicated that there are some patients with RP whose total macular volume and the length of IS/OS line were relatively well preserved in the macular area, but their electro-physiological function within this area was severely affected.

**DISCUSSION**

Our results demonstrated that there was a significant correlation between the amplitudes of the a- and b-waves of the fmERG and the total macular volume in our 43 patients with RP. These results were not surprising because the gradual thinning of the retina caused by the shortening of outer segments and the loss of photoreceptors should result in the reduction of the fmERG amplitude in the retina of patients with RP. The results of an earlier study on the correlation between the retinal histopathology and ERG findings in an animal model of RP support this idea.

Although there was a significant correlation between the amplitude of the fmERG and total macular volume, the degree of correlation was weak: the coefficient of correlation (\(\rho\)) was only 0.46 for the a-wave, and 0.54 for the b-wave. One of the major reasons for this weak correlation was that there were four patients with RP who had normal macular volume but severely reduced fmERG amplitudes (Fig. 4). In contrast, there were no patients with RP who had normal fmERG amplitude but severely reduced total macular volume. These results indicate that a normal total macular volume does not guarantee normal electrophysiological function of the macula in patients with RP.

We initially reasoned that the weak correlation might be because we used total macular volume as a measure of macular structure. It is well known that the early histopathologic changes in eyes of patients with RP were mainly a shortening or distortion of the rod and cone photoreceptors. Thus, we next investigated whether the structural integrity of the IS/OS junction (i.e., the length of the IS/OS line) correlated with the amplitude of the fmERG. As shown, the length of the IS/OS line generally correlated with the fmERG amplitude. However, the correlation between the length of IS/OS line and the fmERG amplitude was also weak. Careful examinations of the OCT images and fmERG records in individual patients with RP.
showed that there were four patients with RP who had normal macular volume and a relatively long IS/OS line, but severely reduced fM Amplitudes (Fig. 6). Of interest, three of these four patients had a detectable IS/OS line longer than 5 mm. These results indicated that there are some patients with RP whose macular OCT images are relatively well preserved, but their electrophysiological functions are severely reduced.

The exact reason that some patients with RP had a preserved macular OCT image but severely reduced fM Amplitude was not determined. There are two possibilities: First, these patients may have very subtle structural changes, but our OCT system (third-generation Stratus OCT) may not have detected the changes. For example, using ultrahigh-resolution OCT, Witkin et al. measured the distance between the IS/OS line and the outer border of the retinal pigment epithelium thickness (called FOSPET), and demonstrated an excellent correlation between visual acuity and FOSPET in nine patients with RP. In our study, we were able to measure the length of the IS/OS line, but could not obtain reliable measurements of FOSPET in our OCT images. New-generation, high-resolution OCT instruments may enable us to make these measurements.

A second possibility is that the functional abnormality may precede structural changes in the macula of some patients with RP. It was recently demonstrated that some patients with Leber congenital amaurosis (LCA), the most common inherited cause of blindness in childhood, can retain the cone photoreceptors and inner retinal architecture in the central retina, but have severely reduced central vision at a relatively early stage of the disease. If this second possibility is correct, the combined assessment of macular structure by OCT and macular function by psychophysics or electrophysiology can provide important information on the macula of patients with RP.

There are some limitations in our study. First, we planned to measure the volume of the inner, middle, and outer retinal layers separately and wanted to examine the correlation between the volumes in each layer and the fM Amplitude. This comparison was possible in normal subjects, but was difficult in patients with RP with severely reduced macular thickness. Recent advances in new ultrahigh-resolution OCT technique may enable analysis of the thickness of each retinal layer, and this will allow us to investigate the changes in each retinal layer after photoreceptor degenerations. Second, we investigated the correlation of macular volume with the fM Amplitude, but did not study the correlation with the implicit time, because there were many patients with RP whose amplitude of fM was so reduced that the implicit time could not
be measured precisely. However, the correlation between the implicit time and OCT images may be another important indicator of functional changes in the macula area of patients with RP. Third, we did not record the OCT and mfERGs from the same patient at different time points, and thus cannot examine the longitudinal progression of the changes in patients with RP.

In conclusion, we studied the correlation between the mfERG amplitude and macular structure by OCT and found that there was a significant correlation between these two measures, but the degree of correlation was weak. One major reason for this low correlation was the presence of some patients with RP who had well-preserved macular OCT images but severely reduced mfERGs. Although the exact mechanism for this discrepancy needs further investigation, we believe that the combined examination of macular structure by OCT and macular function by mfERG can provide important information on the pathophysiology, prognosis, and future treatments in patients with RP.

References

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