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Investigating the Usefulness of Fundus Autofluorescence in Retinitis Pigmentosa

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Purpose: To investigate the relationship between visual field (VF) loss and the rings of the ellipsoid zone (EZ) and autofluorescence (AF) in patients with retinitis pigmentosa (RP).

Design: Cross-sectional study.

Participants: A total of 28 eyes of 15 patients with a clinical diagnosis of RP.

Methods: The VF was measured at a 1-degree interval on the horizontal or vertical lines, within the central 10 degrees from fixation, using MP-3 microperimetry (Nidek Co. Ltd., Aichi, Japan), and the sensitivity deviation from the normative database was calculated. OCT was performed, and the EZ was identified. Fundus AF was also measured, and the AF ring was identified. The VF test points were categorized according to their relationship with the EZ line and AF ring. The relationship between mean retinal sensitivity deviations on both the horizontal and vertical lines and the diameters of the EZ line and the AF ring in the corresponding directions was analyzed. The relationship between visual sensitivity deviation and the locations against EZ line and AF rings was also investigated.

Main Outcome Measures: The relationship between the visual sensitivity deviation and the locations against EZ line and AF rings.

Results: The diameter of the AF ring had a significantly stronger relationship with mean retinal sensitivity deviation compared with the diameter of the EZ line in both the horizontal and vertical directions. Test point location inside/on/outside the AF ring was significantly related to retinal sensitivity deviation independent of the location inside/outside the EZ line ($P < 0.001$, linear mixed model).

Conclusions: Visual sensitivity damage is significantly associated with the EZ lines and AF rings. The development of the AF ring is related to the decrease of visual function independent of and stronger than the EZ line. *Ophthalmology Retina 2018;■:1–9 © 2018 by the American Academy of Ophthalmology*

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Retinitis pigmentosa (RP) comprises progressive retinal disease characterized by night blindness and visual field (VF) defects,[1] which usually start in the peripheral area and gradually progress toward the central area.[2]

Visual field damage in RP eyes is caused by dysfunction and loss of photoreceptors, and dysfunction of the retinal pigment epithelium (RPE).[3] Detailed evaluation of the retina has become possible with the development of spectral-domain OCT (SD-OCT). As a result, the ellipsoid zone (EZ) or photoreceptor inner segment/outer segment junction can now be identified, just internal to the RPE layer. Previous reports have demonstrated a relationship between the EZ and visual function in patients with RP; in particular, the normal alignment of membranous discs, suggested by the presence of a normal EZ line, is important for photoreceptors to maintain function.[4] Indeed, in macular hole surgery, treatment outcome, in terms of visual function, is related to the EZ line integrity.[5-7] In RP, the length of the EZ line is related to mean visual sensitivity,[8,9] and the edge of the EZ corresponds to the point where visual sensitivity decreases sharply.[10,11] Furthermore, the EZ line is also related to best-corrected visual acuity (VA).[12-14] Thus, it is useful to analyze the EZ line when evaluating photoreceptor function in RP.

The distribution of lipofuscin at the RPE, due to deteriorated function of the RPE,[15] can be indicated by a hyperautofluorescent ring (AF ring) in fundus autofluorescence (FAF) imaging of patients with RP.[16-19] It has been suggested that an excessive amount of lipofuscin accumulates in RPE cells as the result of photoreceptor apoptosis and may cause RPE atrophy.[16,20-22] Alternatively, apparent hyperautofluorescence may be due to loss of outer segments because rhodopsin attenuates the signal. Outside the ring, the level of AF is similar to that inside the ring. The half-life of AF is long, such that loss of AF may take many years after loss of photoreceptor cells. After photoreceptor death and RPE atrophy, a hypoautofluorescent area is observed outside the AF ring.[16] Thus, the AF ring suggests the border between the functional and dysfunctional retina.[23] Further, it has been reported that the edge of the AF ring corresponds to the internal border of the VF defect,[19,23] and the diameter of the AF ring is significantly correlated with retinal sensitivity.[24] Disruption of the EZ line and a decrease in the outer nuclear layer thickness have also been reported across

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the AF ring,[25,26] and the diameter of AF ring is significantly related to that of the EZ ring.[24]

Although both the EZ ring and the AF ring are suggestive of damage to visual function, it has also been reported that the diameter of the EZ and the internal diameter of the AF ring are interrelated.[24,27] Few reports have investigated the concurrent effects of the EZ and AF rings on visual function. In particular, it is still unclear whether the appearance of the AF ring is suggestive of additional visual dysfunction beyond the appearance of the EZ ring. In the current study, we carried out SD-OCT and VF measurements in patients with RP. Then, the relationship between VF deterioration and the EZ and AF rings was analyzed simultaneously.

Methods

This study was approved by the Research Ethics Committee of the Graduate School of Medicine and Faculty of Medicine at The University of Tokyo. Written informed consent was given by patients for their information to be stored in the hospital database and used for research. This study was performed according to the tenets of the Declaration of Helsinki.

Participants

This study included 28 eyes of 15 patients with RP. All patients fulfilled the following inclusion criteria: (1) Patients had typical fundus (arteriolar attenuation, retinal pigmentary changes, or waxy disc pallor), reduction in a- and b-wave amplitudes, or nondetectable full-field electroretinography and VF defects corresponding to their fundus; (2) RP was the only disease causing VF damage; (3) patients were followed for at least 6 months at The University of Tokyo Hospital and underwent at least 2 VF measurements before this study; (4) measured best-corrected VA was >20/200; (5) pupil size was >4 mm in diameter, which is required for the MP-3 measurement; and (6) the AF ring was required to be within the central 10 degrees on FAF imaging. Eyes with foveal atrophy, macular edema, or discontinuous EZ line at the fovea were carefully excluded.

OCT Measurement

We used the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) in this study. All OCT images consisted of line scans (horizontal and vertical B-scans). Line scans were created by averaging up to 100 B-scans (768 A-scans per B-scan) within 30°. With these images, the EZ ring was identified and recorded in each patient. Initial identification of the EZ line was performed by an examiner (JL) followed by verification by an independent examiner (SA). If the second estimator did not agree with the first examiner, a panel discussion was held, and the final EZ line was decided. In a similar manner, the lengths of the EZ in the horizontal and vertical lines were also decided.

Fundus Autofluorescence Measurement

Fundus AF imaging was performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2, Heidelberg Engineering). Fundus AF imaging was performed for the 55 degrees field of view. Similar to the identification of EZ ring, initial identification of the external and internal boundaries of the AF ring was performed by an examiner (JL) followed by verification by an independent examiner (SA). If the second estimator did not agree with the first examiner, a panel discussion was held and the final boundaries were decided. In a similar manner, the lengths of boundaries in the horizontal and vertical lines were also decided.

MP-3 Measurement

Each patient had VF testing using the MP-3 microperimeter (Nidek Co. Ltd., Aich, Japan). All patients had a pupil size >4 mm in diameter, which is required for the MP-3 measurement. The MP-3 measurements were performed using the full threshold strategy with the standard Goldmann III stimulus size. Unlike the Humphrey Field Analyzer (Carl Zeiss Meditec AG, Dublin, CA), the MP-3 is equipped with an auto-tracking function; the position of the retina is accurately followed throughout the VF test so that the target stimulus is projected onto a precise location on the retina. The MP-3 also has a wider dynamic range (0−34 decibels [dB]) than its predecessor, the MP-1. The background luminance in the MP-3 is 31.4 asb, which is identical to that in the Humphrey Field Analyzer. We have recently reported that VF sensitivity measured with the MP-3 is associated with significantly better test−retest reproducibility, compared with the Humphrey Field Analyzer, in patients with RP.[28] Also, we have recently reported retinal structure (within or outside EZ) is better discriminated using the MP-3 than with the Humphrey Field Analyzer.[29]

A previous study suggested that high-resolution perimetry with a test point resolution of <1.5 degrees is needed to detect subtle VF defects within the central 10 degrees of patients with glaucoma.[30] The purpose of the current study was to investigate the relationship between retinal structure and VF sensitivity in detail; thus, the VF test sensitivity was measured at every 1 degree; 41 points were allocated at a 1-degree interval on the horizontal and vertical lines, within the central 10 degrees from fixation. The MP-3 examinations were performed in a dim room, similarly to the Humphrey Field Analyzer measurement.

Normal Database

The sensitivity of MP-3 was also measured in 22 normal eyes, so that a normal database was created. The mean age was 49.1 years (standard deviation [SD], 14.3; range, 24−72), 13 right eyes and 8 left eyes. Inclusion criteria for the normal group were (1) no abnormal findings except for clinically insignificant senile cataract on biomicroscopy, gonioscopy, and fundoscopy; (2) no history of ocular diseases that could affect the results of MP-3 examinations, such as diabetic retinopathy or age-related macular degeneration; (3) no abnormal findings on OCT imaging. OCT imaging was carried out in the same manner with patients with RP and reviewed by 2 specialists (ophthalmologists) in macular diseases (TI and RO); and (4) age ≥20 years, spherical equivalent refractive error ≥−6.0 diopters (D) and <3.0 D. This recruitment was carried out irrespective of the status of the fellow eye: The fellow eye could have unilateral macular diseases, such as branch retinal vein occlusion, central serous chorioretinopathy, and age-related macular degeneration. MP-3 measurement was performed in the same manner as patients with RP, and only reliable VFs were used in the analysis using the same definition as in patients with RP.

Superimposing

The OCT and FAF images of each eye were superimposed onto the MP-3 color fundus images using GIMP software (v2.8.20; Free Software Foundation, Inc, Boston, MA). The MP-3 measurement points were categorized according to their location in the EZ ring (inside: EZ_{in} or outside: EZ_{out}) and the internal and external boundaries of the AF ring (inside: AF_{in}, sitting on: AF_{on}, or outside: AF_{out}). The superimposed images of example cases are shown in Figure 1.

Statistical Analysis

First, mean normal MP-3 sensitivity at each test point was calculated. In this calculation, VF sensitivity was regressed against