Correlation of fundus autofluorescence with photoreceptor morphology and functional changes in eyes with retinitis pigmentosa

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ABSTRACT.

Purpose: To assess and correlate fundus autofluorescence (FAF) characteristics with photoreceptor morphology and functional features in eyes with retinitis pigmentosa (RP).

Methods: Thirty-four eyes of 17 patients with RP were examined. We compared FAF images obtained by confocal scanning laser ophthalmoscopy with Spectral-domain optical coherence tomography (SD-OCT) and retinal function assessed by microperimetry.

Results: Normal FAF surrounded by a ring of increased FAF at the macular area was detected in 32 (94%) eyes. The diameter of the normal FAF was correlated significantly with the preserved area of the photoreceptor inner segment and outer segment (IS/OS) junction on SD-OCT ($R = 0.939$, $p < 0.001$). The area outside the ring was associated with loss of IS/OS junction and external limiting membrane (ELM). The ring of increased FAF demarcated the border between the central retina with preservation of the IS/OS junction and ELM, and the adjacent eccentric retina with loss of these bands. In two eyes of one patient, there was no preservation of normal FAF at the macula and the photoreceptor IS/OS junction was not detected on SD-OCT. The mean retinal sensitivity derived from microperimetry was correlated significantly with the area of normal FAF ($R = 0.929$, $p = 0.007$) and the preserved area of the IS/OS junction ($R = 0.851$, $p = 0.032$). Ten eyes had progressive reduction in size of the normal FAF inside the ring accompanied by decreased area of preserved IS/OS during 3.1 years.

Conclusion: FAF appears to reflect the integrity of the photoreceptor layer. It may serve as a secondary outcome measure for novel therapeutic strategies for RP.

Key words: fundus autofluorescence – photoreceptor inner segment and outer segment – retinitis pigmentosa – Spectral-domain optical coherence tomography

Introduction

Retinitis pigmentosa (RP) is a set of inherited disorders characterized by nyctalopia and progressive peripheral visual loss leading to central visual loss. Mutations in a number of photoreceptor-specific genes are associated with RP (Dryja et al. 1990; Brown et al. 2006), and those mutations cause primary degeneration of rod photoreceptors followed by degeneration of cone photoreceptors. Photoreceptor survival seems to correlate closely with visual function (Brown et al. 2006). Therefore, assessment of photoreceptor status may be the most important clinical aspect of evaluating disease progression.

Identification and characterization of photoreceptor degeneration have been well studied in animal models and post-mortem human eyes (Flannery et al. 1989; Porter et al. 1994; Milam et al. 1996; Li et al. 1998; Reme et al. 1998); however, those changes have not been well investigated in vivo because conventional examination such as slit-lamp biomicroscopy and binocular indirect ophthalmoscopy cannot identify those specific changes in detail.

Fundus autofluorescence (FAF) imaging is a noninvasive method of retinal imaging (Delori et al. 1995; von Ruckmann et al. 1995). Originally, the
FAF signal was thought to originate predominantly from lipofuscin in the retinal pigment epithelium (RPE) (Delori et al. 1995). Increased FAF indicates abnormally high metabolic activity, impaired function to degrade waste material from the metabolic activities in the RPE, or the absence of retinal tissue or luteal pigment attenuating the excitation light (Holz et al. 2001; Bessho et al. 2009). While decreased or absent FAF may indicate RPE atrophy, loss of photoreceptors, or the presence of materials that attenuate the signal (Holz et al. 2001; Wakabayashi et al. 2008; Bessho et al. 2009). However, recent studies have demonstrated that the FAF signals reflect not only lipofuscin distribution in the RPE but also other fluorophores in the photoreceptor layer (Sawa et al. 2008; Schmitz-Valckenberg et al. 2008). Because most inherited retinal diseases, including RP, affect mainly the photoreceptors, FAF imaging may provide additional insight into such disease condition.

Spectral-domain optical coherence tomography (SD-OCT) has axial imaging resolution of <5 microns and offers more than 50-fold higher data-acquisition speed compared to conventional time-domain OCT systems (Alam et al. 2006). The improved resolution enhances visualization of intrettinal structures, particularly at the level of the external limiting membrane (ELM) and photoreceptor inner segment/outer segment (IS/OS) junction, which may indicate the integrity of the photoreceptor layer. SD-OCT also provides rapid sweep of serial OCT B-scan images to capture subtle microstructural changes in the area of interest (Hangai et al. 2007; Fujimoto et al. 2008).

The purpose of this study is to obtain FAF images in patients with RP and assess a potential correlation between FAF patterns and photoreceptor morphology obtained by SD-OCT imaging. Changes in the FAF and SD-OCT images over time after initial examination also were followed to explore the possibility of time-dependent changes in the pattern of FAF images and photoreceptor morphology. In addition, retinal function was assessed by microperimetry-1 (MP-1) in selected cases to explore the relation between FAF changes and retinal function.

**Methods**

This study was an observational case series of consecutive patients with RP who were examined at Osaka University Hospital. Diagnoses were based on a history of nyctalopia, characteristic pigimentary retinal changes, bone spicule pigmentation around the periphery, and ISCEV (International Society for Clinical Electrophysiology of Vision) standard full-field ERGs (Marmor et al. 2004). All patients underwent a comprehensive ophthalmologic examination including measurement of the best-corrected visual acuity (BCVA), binocular indirect ophthalmoscopy, contact lens slit-lamp biomicroscopy, and fundus photography. Fundus photographs were obtained using a standard fundus camera (TRC-50LX/ImageNet2000; Topcon, Tokyo, Japan). Patients provided written informed consent after a detailed explanation of the study was provided. Approval from the internal review board was not required for this retrospective study.

FAF images were obtained with a confocal scanning laser ophthalmoscope, Heidelberg Retina Angiograph 2 (HRA2; Heidelberg Engineering, Heidelberg, Germany) as previously described (Wakabayashi et al. 2008). The optically pumped solid state laser (488 nm) was used for excitation, and emission was detected with a barrier filter above 500 nm. The maximum horizontal and vertical diameters of the central normal autofluorescent area were measured by two masked observers with the software included in the HRA2 and the values of the measurements were averaged.

Retinal microstructural imaging was obtained using SD-OCT, RTVue (Optovue Inc., Fremont, CA) as previously described (Fujimoto et al. 2008). Multiple serial cross-sectional images were obtained using a three-dimensional raster scan protocol. The scan consisted of equally spaced 101-frame horizontal B-scans, each comprising 512 A-scans/frame in a transverse direction, covering 4 × 4 mm with a depth of 2 mm. A pair of high-definition 1024 A-scans/frame horizontal and vertical B-scans also was obtained. SD-OCT identifies three distinct lines corresponding to back reflection from the ELM, IS/OS junction, and RPE/Bruch’s membrane. Microstructural changes in the photoreceptor layer were defined as loss of the back-reflection line corresponding to the photoreceptor IS/OS junction or ELM. The extent of the preserved photoreceptor IS/OS junction in the SD-OCT images was measured on the horizontal and vertical scans through the centre of the fovea by two masked observers using software in the RTVue and the values of the measurements were averaged. To assess the potential correlation of FAF images with photoreceptor morphology on SD-OCT, the virtual OCT fundus image created by the raster scan protocol was correlated with FAF image and used for the registration of each cross-sectional OCT image to the specific features on the FAF image.

Microperimetry was performed with automatic fundus-related perimetry (Nidek Technologies, Padova, Italy) in selected patients who agreed with this examination. Stimuli attenuation ranged from 0 to 20 decibels (dB). Stimulation size was equivalent to the Goldmann III spot size, with 200-ms projection; the fixation target was a 2-degree cross. The ‘4-2 strategy’ default setting was applied, and 56 stimulus locations covering the central 16 degrees were examined.

After the initial enrolment, eyes with pre-existing macular disease, cystoid macular oedema (CME), cataracts, or diabetes mellitus, which could affect VA measurement or interfere with image interpretation, were excluded from data analysis. For statistical analysis, the BCVA was measured using the Landolt C acuity chart and analysed on a logarithm of minimal angle of resolution (logMAR) scale; values of 2.0 and 3.0 indicated counting fingers and hand motions vision, respectively. To assess the inter-observer repeatability of the measurements of normal autofluorescent area on FAF images and preserved photoreceptor IS/OS area on SD-OCT images, the methods described by Bland and Altman was used (Bland & Altman 1986). The mean difference between two measurements (Observer1–Observer2) for each of the FAF and SD-OCT images represented the bias. The 95% limits of agreement (LoA), an expected difference between two measurements, was calculated as the mean of the differences ± 1.96 × standard deviation (SD) of the difference. The coefficient of
indicated statistical significance. (SPSS Inc., Chicago, IL). p < 0.05 using SigmaStat software version 3.1.

Results

Basic information

Forty-four eyes of 22 consecutive patients with RP underwent examinations. Ten eyes were excluded because of CMO (two eyes of one patient) and cataracts (eight eyes of four patients), leaving 34 eyes of 17 patients (9 men, 8 women; mean age, 39.8 ± 18.3 years; range, 12–80) for data analysis. The mean BCVA was 0.55 (range, hand motions-1.5). The patients had the typical bone spicule appearance in the peripheral retina. The posterior pole had a relatively normal appearance.

FAF images

The most prominent feature of FAF in most patients (32/34 eyes; 94%) was a ring of increased autofluorescence in macular area with different eccentricities (Figs 1–4). The normal FAF was observed inside the ring in those 32 eyes. Outside the ring, various FAF patterns were seen, i.e., mottled decreased FAF in eight eyes (4 patients) (Fig. 1), apparently normal FAF in four eyes (2 patients) (Fig. 4D), and apparently normal FAF surrounded by mottled decreased FAF in 20 eyes (10 patients) (Figs 2, 3 and 4A). The mean diameters of the normal FAF surrounded by the hyperfluorescent ring on horizontal and vertical sections through the fovea were 1.71 ± 1.29 (range, 0.43–5.60) mm and 1.23 ± 1.23 (range, 0.34–5.86) mm, respectively. The mean diameter of the normal FAF on horizontal and vertical sections was significantly associated with VA (linear regression analysis, R = 0.37, p = 0.03).

Two eyes of one patient did not have a ring as well as the normal FAF in the macular area, and a small area of increased FAF was seen centrally (diameters, 1340 μm and 1360 μm in the right eye, and 1310 μm and 1110 μm in the left eye on horizontal and vertical sections) (Fig. 4G). The VA levels in these eyes were the worst among the study eyes (0.2 in the right eye and hand motions in the left eye).

SD-OCT images

Based on multiple serial B-scans, the photoreceptor IS/OS and ELM were preserved in a different degree at the macular area in 32 eyes with hyperfluorescent rings. In two eyes of one patient, the photoreceptor IS/OS was not seen at the macular area. In 32 eyes with a hyperfluorescent ring, the interface between the preserved and lost IS/OS was seen clearly. The mean extent of the preserved IS/OS junction was 1.90 ± 1.23 mm (range, 0.49–5.26) and 1.73 ± 1.18 mm (range, 0.44–5.39) on horizontal and vertical B-scans, respectively. The transverse extent of the preserved ELM was larger than that of the IS/OS in the 32 eyes (0.83 ± 0.45 and 0.77 ± 0.39 larger, on horizontal and vertical sections, respectively). Outside the intact ELM, the photoreceptor IS/OS and ELM were not seen even in eyes in which the retina appeared relatively normal. The diameter of preserved IS/OS on the horizontal and vertical sections was significantly associated with VA (R = 0.421, p = 0.013 and R = 0.399, p = 0.019, respectively).

Repeatability of the analysis

The mean difference in horizontal and vertical diameters of the normal FAF area between observer1 and 2 were + 0.02 mm and + 0.03 mm, respectively. No significant bias was found between observers (p = 0.953, p = 0.837, respectively). The 95% LoA were between −0.23 and 0.27 mm in the horizontal measurement and −0.23 and 0.28 in the vertical measurement. Therefore, the coefficient of repeatability was 0.24 in the horizontal section and 0.25 in the vertical section. The bias of the measurements for preserved IS/OS area in the SD-OCT images was 0.00 mm with 95% LoA between −0.19 and 0.20 mm in the horizontal measurement and −0.02 mm with 95% LoA between −0.17 and 0.20 mm. No significant bias was found between observers (p = 0.883, p = 0.352, respectively).
Correlation of FAF with SD-OCT images

The area of normal FAF surrounded by the hyperfluorescent ring was corresponded to the area of preserved IS/OS on SD-OCT (Figs 1–4). The diameter of the normal FAF at the macular area was significantly correlated to the preserved area of the IS/OS on SD-OCT horizontally and vertically ($R = 0.939$, $p < 0.001$ and $R = 0.962$, $p < 0.001$, respectively). No IS/OS was seen in areas with a ring of increased FAF; however, the ELM was preserved in those areas. Outside the areas with a ring of increased FAF, both IS/OS and ELM were not observed and there was gradual thinning of the outer nuclear layer towards the periphery. All eyes with hyperfluorescent rings had no IS/OS and ELM outside the ring, regardless of the FAF pattern outside the ring. Therefore, the ring of increased FAF demarcated the border between the central retina with preserved IS/OS and ELM and the adjacent peripheral retina with no IS/OS and ELM. In two eyes of one patient without normal FAF at the macula, the IS/OS was not seen on SD-OCT even at the fovea (Fig. 4C).

Microperimetry

Microperimetry was performed in six eyes. Retinal sensitivity was detected only within the hyperfluorescent ring on FAF (Figs 1 and 3); the mean retinal sensitivity within the examined area was $4.39 \pm 2.40$ dB (range, 1.04–6.64). The mean retinal sensitivity was correlated significantly with the area of normal FAF seen by HRA2 ($R = 0.929$, $p = 0.007$) and the preserved area of the IS/OS on SD-OCT ($R = 0.851$, $p = 0.032$).

Time-dependent changes in FAF and SD-OCT images

Of the 32 eyes with a hyperfluorescent ring, 26 eyes (81%) continuously underwent FAF and SD-OCT examinations during 3.1 (range, 3.3–5) years of follow-up to determine the possibility of time-dependent changes in FAF and SD-OCT images. Ten eyes from 5 patients had progressive reduction in size of the ring as well as the area of normal FAF inside the ring. Those changes were accompanied by decreased area of preserved IS/OS. One of these patients had disappearance of pre-existing normal FAF with concomitant loss of intact IS/OS (Fig. 5). In 16 eyes of 8 patients, the normal FAF inside the ring and associated preserved IS/OS showed no significant changes during the follow-up.

Discussion

In the current study, the FAF images in patients with RP showed various sizes of characteristic hyperfluorescent ring and the normal FAF inside the ring. The area of normal FAF measured with acceptable repeatability was significantly correlated with a preserved area of photoreceptor IS/OS junction on SD-OCT as previously reported (Murakami et al. 2008; Lima et al. 2009). The intact photoreceptor morphology with normal metabolic activities may lead to normal lipofuscin distribution in the RPE, resulting in normal intensity of the FAF. In addition, the area of normal FAF had preserved retinal functions based on the microperimetric examination. Therefore, the area of normal FAF inside the ring may be the key for evaluating the integrity of photorecept-
tor IS/OS as well as the visual function in RP (Robson et al. 2003, 2004, 2006; Popovic et al. 2005). The normal FAF with a larger diameter was also significantly associated with a better VA in our series.

Outside the ring of increased FAF, the FAF images showed various patterns, i.e., mottled decreased FAF in eight eyes (Fig. 1), apparently normal FAF in four eyes (Fig. 4D), and apparently normal FAF surrounded by mottled decreased FAF in 20 eyes (Figs 2, 3 and 4A). However, the patterns outside the ring were less relevant for evaluating the visual function because both photoreceptor IS/OS and ELM were completely lost and the retinal function was not maintained regardless of the pattern of FAF outside the ring. Only the area of normal FAF inside the ring seems to be clinically relevant for evaluating the visual function.

The reason why some eyes present normal intensities of the FAF despite photoreceptor loss outside the ring is not clear in the present study. However, these findings may be similar to the findings of normal-appearing FAF with photoreceptor loss outside the ring observed in patients with pigmented paravenous retinochoroidal atrophy which Fleckenstein et al. reported. They considered the normal-appearing FAF without intact photoreceptor layer represented surviving RPE containing lipofuscin granules that were formed prior to the occurrence.

Fig. 4. Various patterns of fundus autofluorescence (FAF) and Spectral-domain optical coherence tomography (SD-OCT) images. (A) An FAF image from a 54-year-old woman shows an area of relatively large normal FAF including the macula. A ring of increased FAF around the normal FAF can be seen. Outside this, apparently normal FAF surrounded by an area of dark mottling is detected. The white line indicates the locations of SD-OCT image shown in panel B. (B) A horizontal cross-sectional SD-OCT image. (C) Magnification of the area outlined in B shows a largely preserved photoreceptor IS/OS junction within areas with normal FAF. A cross-sectional image scan shows the absence of the IS/OS junction and external limiting membrane (ELM) outside the ring with apparently normal FAF surrounded by dark mottling. (D) An FAF image from a 15-year-old girl shows normal FAF centrally surrounded by relatively large areas of increased FAF. Outside this, apparently normal FAF is seen. The white line indicates the locations of SD-OCT image shown in panel E. (E) A horizontal cross-sectional SD-OCT image. (F) Magnification of the outlined area in E shows a preserved IS/OS junction within areas with normal FAF. The IS/OS junction and ELM are lost outside area of increased FAF, although the FAF has apparently normal intensity. (G) An FAF image from a 35-year-old man shows increased FAF centrally without normal FAF at the macula. The white line indicates the locations of SD-OCT image shown in panel H. (H) A horizontal cross-sectional SD-OCT image. (I) Magnification of the area outlined in H shows loss of the IS/OS junction.

Fig. 5. Changes in the Fundus autofluorescence (FAF) and SD-OCT images of a 56-year-old man. (A) An FAF image of the right eye shows normal FAF centrally surrounded by a ring of increased FAF. Outside this, decreased FAF with a mottled appearance is present. (B) A horizontal cross-sectional SD-OCT image shows an intact photoreceptor IS/OS junction within areas with normal FAF. The IS/OS junction and external limiting membrane (ELM) are absent outside the ring. (C) After three years, the FAF image showed reduction in size of the ring as well as the area of normal FAF inside the ring. (D) The area of preserved IS/OS decreased. (E) After six months, the pre-existing normal FAF disappeared. (F) The SD-OCT image shows loss of intact IS/OS. The ELM seems to be preserved.
rence of photoreceptor impairment. Normal FAF in the presence of severe photoreceptor dysfunction also has been reported in patients with leber congenital amaurosis (Scholl et al. 2004a). Although photoreceptor morphology in the normal FAF were not investigated in their study, the normal metabolic activities of the photoreceptors and constant outer segment phagocytosis by the RPE may not be necessarily required for normal FAF in some patients with inherited retinal dystrophies.

The increased FAF has been reported to indicate dysfunction of the RPE cells and represent reduced retinal sensitivity. (Delori et al. 1995; von Ruckmann et al. 1995; Scholl et al. 2004b). A pathologic RPE might not digest visual pigment from the photoreceptors, resulting in excess accumulation of autofluorescent materials and subsequent increased FAF, as suggested in patients with age-related macular degeneration (AMD) (Holz et al. 1999). However, recent studies have reported that the FAF represents the condition of not only the RPE but also photoreceptors in various retinal diseases (Sawa et al. 2008; Schmitz-Valckenberg et al. 2008). Because the primary disease locus is the photoreceptors in most RP cases, the hyperfluorescent ring in patients with RP may also be related to the photoreceptors. In our series, IS/OS was not observed in areas with a ring, although the ELM was present. Those areas of hyperfluorescent ring might represent regional distribution of active photoreceptor degeneration and the increased rate of outer segment phagocytosis by the RPE.

Histopathologic studies of RP have shown that the shortening of the rod outer segments and reduction of the cell bodies indicative of photoreceptor cell death occur at the midperiphery and progress with time to involve the cone photoreceptors (Flannery et al. 1989; Portera-Cailliau et al. 1994; Milam et al. 1996; Li et al. 1998; Reme et al. 1998). These findings may be compatible with the time-dependent changes in the FAF and SD-OCT images in the current series, which Robson et al. 2006 first reported. They identified the constriction of the ring of increased FAF and associated worsening of macular function in RP. In the current series, ten eyes had reduction in size of the ring as well as the area of normal FAF inside the ring within three and a half years. Although the morphologic implications of the progressive ring constriction were not investigated further in their study, we newly found that the reduced size of normal FAF was accompanied by decreased area of intact photoreceptor IS/OS (Fig. 5). The photoreceptor death may progress to involve the outermost region of the normal FAF inside the ring, resulting in constriction of the ring and reduced retinal function. With the progressive replacement of normal FAF with the constricted ring, the island of normal FAF inside the ring eventually disappear, resulting in increased FAF centrally without normal FAF and IS/OS, as demonstrated in one patient at the initial examination with worst visual function (Fig. 4C) and another patient during the follow-up (Fig. 5).

In contrast to the eyes with ring constriction, sixteen eyes had no significant changes in the area of normal FAF inside the ring and IS/OS during the follow-up. This finding may suggest that the disease was stationary or had limited progression. The presence or absence of the ring constriction during the follow-up may reflect the difference in the rate of photoreceptor death in the studied eyes. Therefore, the areas of normal FAF inside the ring and the rate of subsequent ring constriction seems critical for predicting the visual prognosis. Further studies are needed to identify the possible factors that may influence the rate of ring constriction, such as patient age, mode of inheritance or gene mutation.

In summary, FAF and SD-OCT are useful objective tools for evaluating the photoreceptor status and the time course of progressive photoreceptor loss not seen on clinical examination in patients with RP. Normal FAF surrounded by a hyperfluorescent ring indicates the integrity of the photoreceptor IS/OS and the ELM with preserved retinal function. Long-term preservation of the normal FAF to maintain visual function may be an objective in the development of new therapeutic strategies in the future.

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Changes of fundus autofluorescence, photoreceptor inner and outer segment junction line, and visual function in patients with retinitis pigmentosa

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ABSTRACT

Background: To determine whether a significant correlation exists among the changes in the size of the abnormal parafoveal autofluorescence ring, the length of the photoreceptor inner and outer segment (IS/OS) junction, and the visual function in patients with retinitis pigmentosa.

Methods: Retrospective observational case series. A total of 50 eyes of 26 patients with typical retinitis pigmentosa and an autofluorescence ring were examined by optical coherence tomography and microperimetry. During the follow-up period of >2 years, the changes in the diameter and area of the autofluorescence ring, the length of the IS/OS line, the best-corrected visual acuity and mean retinal sensitivity in the central 10° were determined.

Results: The diameter and area of the autofluorescence ring, and the length of the IS/OS line decreased significantly during the follow-up period (all, P < 0.0001). The decrease in autofluorescence ring diameter was significantly correlated with the decrease in retinal sensitivity, visual acuity and IS/OS length (P = 0.0105, P = 0.0252 and P < 0.0001, respectively). The decrease in autofluorescence ring area was significantly correlated with the decrease in retinal sensitivity, visual acuity and IS/OS length (P < 0.0001, P = 0.0026 and P = 0.0011, respectively).

Conclusion: During the progression of retinitis pigmentosa, the progressive constriction of the autofluorescence ring reflects the morphological changes of the photoreceptors, and is associated with a worsening of visual function.

Key words: fluorescence, optical coherence tomography, retinal photoreceptor cell outer segment, retinitis pigmentosa, visual acuity.

INTRODUCTION

Fundus autofluorescence (FAF) results from the accumulation of lipofuscin in the retinal pigment epithelial (RPE) cells, and has been used to investigate retinal diseases.1-4 Several patterns of the FAF have been observed in patients with retinitis pigmentosa (RP), and more than half of the RP patients have an abnormally high-density parafoveal FAF ring (AF ring).5 Robson et al.5 reported that the AF ring represents the border between functional and dysfunctional retina, whereas Popovic et al.3 found that the radius of the AF ring was strongly correlated with retinal sensitivity. In one case of RP, the size of the AF ring was found to decrease accompanied by increasing macular dysfunction.2 However, the details of the changes in AF ring in RP patients has
not been reported, and whether there is a significant correlation between the changes in the AF ring and visual function has also not been reported.

The earliest histopathological changes in the photoreceptors of eyes with RP is a shortening of the photoreceptor outer segments. The loss of the cones reduces central vision at the end stage of the disease. Therefore, morphological assessments of the photoreceptors in the macular area can be useful way to estimate the residual visual function in RP patients objectively.

Optical coherence tomography (OCT) is a well-established method of examining the retinal architecture in vivo. The junction of the photoreceptor inner/outer segment (IS/OS) is observed in OCT images as a distinct, highly reflective line just vitread to the RPE. A distinct and continuous IS/OS line has been shown to indicate a normal alignment of the membranous discs in the photoreceptor outer segments. Because an alignment of the discs is necessary for the normal functioning of the photoreceptors, a continuous IS/OS line would strongly suggest that the photoreceptors are functioning normally. We have reported that the presence of the IS/OS line in the OCT images was correlated with the recovery of good vision after surgery for a macular hole or an epiretinal membrane. In RP patients, several OCT studies have been performed to determine whether a significant correlation exists between the presence and continuity of the IS/OS line and the visual function. Sandberg et al. found a significant correlation between the grade of the IS/OS line and the visual acuity in RP patients. We have reported that the length of the IS/OS line was significantly correlated with the retinal sensitivity and the visual acuity in RP patients. However, a correlation between the changes of IS/OS line and the changes in the visual function during the progression of RP has not been determined.

Thus, the purpose of this study was to evaluate the changes of AF ring and IS/OS line in relation to visual function during the progression of RP.

**METHODS**

We first review all our hospital notes for typical RP, AF ring and follow-up period above 2 years; and these three criteria were general inclusion criteria. The clinical diagnosis of RP was made based on the clinical history, funduscopic appearance, visual field testing, fluorescein angiography and International Society for Clinical Electrophysiology of Vision standard full-field electroretinograms consistent with rod–cone dystrophy. Typical RP was defined RP excluding atypical RP, such as sector RP and unilateral RP. This retrospective observational case study was done on 50 eyes of 26 consecutive patients (14 women and 12 men) who were diagnosed with typical RP, and had an AF ring and a follow-up period of more than 2 years. We took FAF images in 109 eyes of 57 patients with typical RP, and an AF ring was observed in 63 eyes (57.8%) of 33 patients. In these 33 patients, seven patients did not have a follow-up period of more than 2 years. As a result, the other 26 patients met the inclusion criteria of the present study. Eyes were excluded if they had an epiretinal membrane, macular oedema, poor fixation because of low visual acuity, myopia with a posterior staphyloma and cataract that covered the centre of the lens and affected the visual acuity. In 52 eyes of the 26 patients, two eyes were excluded because of posterior staphyloma or cataract. The age of the patients ranged from 23 to 76 years with a median of 54 years (interquartile range: 18.75). The follow-up period ranged from 24 to 47 months with a median of 33 months (interquartile range: 16).

This study was conducted at Chiba University Hospital between August 2005 and July 2009. All subjects underwent a standard ophthalmological examination, including best-corrected visual acuity (BCVA) measurements, applanation tonometry, slit-lamp biomicroscopy, indirect ophthalmoscopy and colour fundus photography. The BCVA was measured with a Japanese standard Landolt visual acuity chart and converted to the logarithm of the minimal angle resolution (logMAR) units for statistical analyses. Electrophysiological examinations other than full-field electroretinograms were not performed.

The procedures conformed to the tenets of the Declaration of Helsinki; and an approval for the protocol of this study was obtained from the Institutional Review Board of Chiba University. An informed consent was obtained from each patient before enrolment in this study.

The FAF images were taken with the Topcon TRC 501X fundus camera (Tokyo, Japan). This fundus camera-based system used a band pass filter centred at 580 nm (bandwidth, 500–610 nm) for the excitation light and a matched barrier filter centred at 695 nm (bandwidth, 675–715 nm; Spectrotech, Saugus, MA, USA) for the observation light. The gain for the digital camera was fixed at 250. The diameter and area of AF ring were calculated using ImageNet (Topcon, Tokyo, Japan); and the average of three measurements was used for the analyses (Figs 1a–d, 2, 3a,b). The diameter of the AF ring was defined as the horizontal dimension of the inner border of the AF ring that passed through the fovea, because the inner border is usually well demarcated in contrast to the outer border. The vertical diameter of the AF ring was not calculated. The masked investigators drew a line at the inner part of the AF ring to calculate the AF ring area.

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Tomographic images of the retina were obtained with the OCT3 (Zeiss Humphrey, Sun Leandro, CA, USA) from 5-mm horizontal scans through the fovea through a dilated pupil. The grayscale images were used for a more precise identification and measurement of the IS/OS line (Figs 1e,f, 3c,d). The length of the IS/OS line that was detected just beneath the fovea was measured three times and averaged. The diameter and area of the AF ring and the length of the IS/OS line were measured independently by two of the authors (AH and TS) who were masked to the other findings and data (including follow-up images) in the patients. In the event of disagreement, a third investigator (YM) was consulted for the final determination. The FAF and OCT data were analysed only at the baseline and final visit.

Microperimetry was performed with the MP-1 (Nidek, Gamagori, Japan) using the software available with the 2006 model (Version: MP1 SW 1.4.1. SP1) with automated correction for eye movements. A 4-2-1-staircase strategy with Goldmann III size stimulus was used; and 24 stimulus locations covering the central 10° were examined (Fig 3e,f). The mean retinal sensitivity for the 24 locations was calculated.

For the statistical analyses, the decrease in the diameter and area of the AF ring, the length of the IS/OS line and the retinal sensitivity was defined as the value at the final visit subtracted from the value obtained at the baseline. The decrease in BCVA was defined as the BCVA in logMAR units at the baseline subtracted from the logMAR BCVA obtained at the final visit.
Wilcoxon signed ranks test were used to determine the significance of the changes in the AF ring, the length of the IS/OS line, the BCVA and the retinal sensitivity. The correlation of the changes in the diameter and area of the AF ring, length of the IS/OS line, BCVA and retinal sensitivity was determined by calculating the Spearman’s rank correlation test. A $P$-value $<0.05$ was considered statistically significant.

**RESULTS**

The diameter and area of the AF ring, length of the IS/OS line, BCVA and retinal sensitivity at the baseline and final visit are presented in Table 1. The diameter and area of the AF ring, length of the IS/OS line, BCVA and retinal sensitivity decreased or were unchanged at the final visit in all of the 50 eyes. The values of these parameters had decreased significantly at the final examination (all, $P < 0.0001$; Wilcoxon signed ranks test; Figs 4–6).

The decrease in AF ring diameter was significantly correlated with the decrease in retinal sensitivity, BCVA and IS/OS length ($r = 0.403$, $P = 0.0105$; $r = 0.219$, $P = 0.0252$; $r = 0.686$, $P < 0.0001$, respectively; Spearman’s rank correlation test; Table 2, Fig. 7). The decrease in AF ring area was significantly corre-
translated with the decrease in retinal sensitivity, BCVA and IS/OS length \((r = 0.594, P < 0.0001; r = 0.405, P = 0.0026; r = 0.419, P = 0.0011, \text{respectively}; \text{Spearman’s rank correlation test}; \text{Table 2, Fig. 8}).

Figure 4. Box plot of the diameter of the autofluorescence ring at the baseline and final visit. The autofluorescence ring diameter had decreased significantly at the final visit \((P < 0.0001)\). The boundaries of each box indicate the 25th and 75th percentiles, the whiskers indicate the minimum and maximum values, and the line within each box indicates the median.

Figure 5. Box plot of the area of the autofluorescence ring at the baseline and final visit. The autofluorescence ring area had decreased significantly at the final visit \((P < 0.0001)\). The boundaries of each box indicate the 25th and 75th percentiles, the whiskers indicate the minimum and maximum values, and the line within each box indicates the median.

Figure 6. Box plot of the length of the photoreceptor inner/outer segment junction (IS/OS) line at the baseline and final visit. The IS/OS length had decreased significantly at the final visit \((P < 0.0001)\). The boundaries of each box indicate the 25th and 75th percentiles, the whiskers indicate the minimum and maximum values, and the line within each box indicates the median.

Table 2. Correlation of decrease in AF ring, IS/OS, BCVA and retinal sensitivity

<table>
<thead>
<tr>
<th>Correlation</th>
<th>r value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in AF ring diameter (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal sensitivity decrease (dB)</td>
<td>0.403</td>
<td>0.0105</td>
</tr>
<tr>
<td>BCVA decrease (logMAR)</td>
<td>0.219</td>
<td>0.0252</td>
</tr>
<tr>
<td>Decrease in IS/OS length (mm)</td>
<td>0.686</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Decrease in AF ring area (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal sensitivity decrease (dB)</td>
<td>0.594</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BCVA decrease (logMAR)</td>
<td>0.405</td>
<td>0.0026</td>
</tr>
<tr>
<td>Decrease in IS/OS length (mm)</td>
<td>0.419</td>
<td>0.0011</td>
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<tr>
<td>Decrease in IS/OS length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal sensitivity decrease (dB)</td>
<td>0.458</td>
<td>0.0018</td>
</tr>
<tr>
<td>BCVA decrease (logMAR)</td>
<td>0.142</td>
<td>0.0291</td>
</tr>
</tbody>
</table>

AF ring, autofluorescence ring; BCVA, best-corrected visual acuity; IS/OS, photoreceptor inner/outer segment junction.

The decrease in IS/OS length was significantly correlated with the decrease in retinal sensitivity and BCVA \((r = 0.458, P = 0.0018; r = 0.142, P = 0.0291, \text{respectively}; \text{Spearman’s rank correlation test}; \text{Table 2}).

At the baseline, the diameter of the AF ring was significantly correlated with the IS/OS length and retinal sensitivity \((r = 0.854, P < 0.0001; r = 0.612, P < 0.0001, \text{respectively but not with the logMAR BCVA}; (r = -0.210, P = 0.2017; \text{Spearman’s rank correlation test}). The area of the AF ring was
significant correlation with the IS/OS length, retinal sensitivity and logMAR BCVA \( (r = 0.821, P < 0.0001; r = 0.588, P < 0.0001; r = -0.282, P = 0.0492, \text{ respectively; Spearman’s rank correlation test}) \). At the baseline, the length of the IS/OS line was significantly correlated with the retinal sensitivity and logMAR BCVA \( (r = 0.786, P < 0.0001; r = -0.285, P = 0.0289; \text{Spearman’s rank correlation test}) \).

In 24 patients in which both eyes were included in this study, the symmetry between eyes of the same patient was examined. The decrease in the diameter and area of the AF ring, the length of the IS/OS line, retinal sensitivity and BCVA in the right eye were not significantly correlated with these parameters in the left eye \( (r = 0.111, P = 0.4392; r = -0.045, P = 0.4862; r = 0.121, P = 0.2696; r = -0.105, P = 0.8724; r = -0.047, P = 0.4797, \text{respectively; Spearman’s rank correlation test}) \).

The relationship between the AF ring diameter and correlation of the decrease in the AF ring diameter and BCVA was examined. In 25 eyes with baseline AF ring diameter of 1.35 mm or less, the decrease in AF ring diameter was significantly correlated with the decrease in BCVA \( (r = 0.378, P = 0.0237; \text{Spearman’s rank correlation test}) \). In the other 25 eyes with baseline AF ring diameter of more than 1.35 mm, however, the decrease in AF ring diameter was not significantly correlated with the decrease in BCVA \( (r = -0.032, P = 0.8713; \text{Spearman’s rank correlation test}) \).

**DISCUSSION**

Lipofuscin accumulates in lysosomes as a byproduct of the photoreceptor outer segment renewal.\(^{17}\) Normal or near-normal FAF indicates the presence of structurally intact photoreceptors and the integrity of the photoreceptor/RPE complex.\(^{17}\) Normal photoreceptor function is dependent on normal RPE cell function and its contribution to the visual cycle and the constant phagocytosis of shed distal outer segments, a process involved in the renewal of photoreceptor discs. Changes in RPE function in RP patients may be a secondary phenomena that can result from increased metabolic load on the RPE due to photoreceptor apoptosis.\(^{3,18}\) Abnormal hyperfluorescence that is seen in the AF ring may indicate an abnormally high rate of phagocytosis of degraded photoreceptors.\(^{19}\) When the lipofuscin accumulation reaches a critical level, which would cause an FAF signal close to the maximum intensity, the RPE cells die with a concomitant loss of lipofuscin granules. The death of photoreceptor cells with RPE atrophy leads to the absence of FAF\(^3\) that can be seen in the peripheral area. Murakami et al.\(^{19}\) also suggested that photoreceptor death may occur first with subsequent RPE degeneration in RP patients with the AF ring. However, clinicopathologic analysis is needed to determine whether a precise association exists between the AF ring and the actual photoreceptor status.

The AF ring represents a transition between abnormal paracentral and normal central cone system function and corresponds to the inner margin of the visual field defect.\(^{2–4}\) In our patients, the AF ring diameter was significantly correlated with retinal sensitivity at the baseline. Robson et al.\(^2\) reported that the size of the AF ring showed progressive constriction in 3 of 12 cases with RP after 2–5 years. One of these three cases underwent serial visual field and pattern electroretinograms testing, and was accompanied by increasing macular...
dysfunction. In the other 9 of their 12 cases, no detectable change was observed. However, they did not measure the diameter and area of the AF ring. Thus, there was a possibility that a slight constriction of the AF ring was missed. Our results showed that the size of the AF ring progressively constricted accompanied by IS/OS shortening, decrease in retinal sensitivity and BCVA worsening. The decrease in AF ring diameter was significantly correlated with the decrease in retinal sensitivity and visual acuity. Moreover, the decrease in AF ring area was significantly correlated with the decrease in retinal sensitivity and visual acuity. These results indicated that the progressive constriction of the AF ring may reflect a worsening of visual function during the progression of RP.

Fleckenstein et al.\textsuperscript{19} and Lima et al.\textsuperscript{21} reported that IS/OS disruption was found across the AF ring using spectral-domain OCT. It has been reported that the diameter of the AF ring was significantly correlated with the IS/OS length in 20 patients with RP.\textsuperscript{19} Our finding that the diameter and area of the AF ring were significantly correlated with the IS/OS length at the baseline is in agreement. We also found that the decrease in the diameter and area of the AF ring was significantly correlated with the decrease in the length of the IS/OS line. This indicates that a progressive constriction of the AF ring may reflect the morphological changes of the photoreceptors during the progression of RP. However, we used time-domain OCT instead of spectral-domain OCT in this study. Therefore, more precise OCT findings using spectral-domain OCT should be investigated in the future.

In eyes with small AF ring, the decrease in AF ring diameter was significantly correlated with the decrease in BCVA. In eyes with large AF ring, however, the decrease in AF ring diameter was not significantly correlated with the decrease in BCVA. This result indicates that a positive correlation between the decrease of AF ring diameter and BCVA is present only in eyes in which the centre of the fovea starts to be involved.

One of limitations of this study was the differences in the follow-up period. A second limitation of this study was that we had no data of electrophysiological changes and used time-domain OCT instead of spectral-domain OCT. We could not ensure that the OCT scan was placed through the same location at the final visit as compared with the baseline examination, because we used time-domain OCT. A third limitation of this study was that we studied the FAF and IS/OS line only in eyes with an AF ring. Murakami et al.\textsuperscript{19} classified the eyes of RP patients according to three types of abnormal FAF: (i) those with an AF ring; (ii) those with abnormal central AF, that is, high-density disciform FAF spreading centrifugally from the fovea; and (iii) the absence of both patterns. Murakami et al.\textsuperscript{19} reported that the AF ring was detected in 59%, the abnormal central AF in 18% and the absence of both patterns in 24%. Our finding that the AF ring was observed in 63 (57.8%) of 109 eyes with typical RP is in agreement. Although most of the RP patients have an AF ring, changes of FAF and IS/OS in eyes without AF ring should be investigated in the future.

In summary, our results showed that the size of AF ring decreased with the progression of RP. This was accompanied by a shortening of the length of the IS/OS line, a decrease in retinal sensitivity and a worsening of the BCVA. The decrease in diameter of the AF ring was significantly correlated with the decrease in retinal sensitivity, visual acuity and IS/OS length. Moreover, the decrease in area of the AF ring was significantly correlated with the decrease in retinal sensitivity, visual acuity and IS/OS length. These results indicate that a progressive AF ring constriction may reflect morphological changes of the photoreceptors and worsening of visual function in the progression of RP. Thus, the AF ring and the IS/OS line may be important parameters to monitor in RP patients.

References

8. Baba T, Yamamoto S, Arai M et al. Correlation of visual recovery and presence of photoreceptor inner/outer


