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STRUCTURAL ASSESSMENT OF HYPERAUTOFLUORESCENT RING IN PATIENTS WITH RETINITIS PIGMENTOSA

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Abstract

Purpose—To analyze the retinal structure underlying the hyperautofluorescent ring visible on fundus autofluorescence in patients with retinitis pigmentosa.

Methods—Twenty-four eyes of 13 patients with retinitis pigmentosa, aged 13 years to 67 years, were studied. The integrity of the photoreceptor cilia, also known as the inner/outer segment junction of the photoreceptors, the outer nuclear layer, and retinal pigment epithelium, was evaluated outside, across, and inside the ring with spectral-domain optical coherence tomography (OCT).

Results—Inside the foveal area, fundus autofluorescence did not detect abnormalities. Outside the ring, fundus autofluorescence revealed hypoautofluorescence compatible with the photoreceptor/retinal pigment epithelium degeneration. Spectral-domain OCT inside the ring, in the area of normal foveal fundus autofluorescence, revealed an intact retinal structure in all eyes and total retinal thickness values that were within normal limits. Across the ring, inner/outer segment junction disruption was observed and the outer nuclear layer was decreased in thickness in a centrifugal direction in all eyes. Outside the hyperautofluorescent ring, the inner/outer segment junction and the outer nuclear layer appeared to be absent and there were signs of retinal pigment epithelium degeneration.

Conclusion—Disruption of the inner/outer segment junction and a decrease in outer retinal thickness were found across the central hyperautofluorescent ring seen in retinitis pigmentosa. Outer segment phagocytosis by retinal pigment epithelium is necessary for the formation of an hyperautofluorescent ring.

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Keywords

cilia; fundus autofluorescence; inner segment; optical coherence tomography; outer segment; retinitis pigmentosa

Retinitis pigmentosa is a group of inherited retinal degenerations characterized by night blindness, progressive peripheral visual field defects, and eventually legal blindness.¹ Visual loss in retinitis pigmentosa (RP) results from the death of photoreceptors and retinal pigment epithelium (RPE) cells.^{2,3} This degenerative process observed in most patients with RP is related to primary deterioration of the photoreceptors cells themselves, which in turn leads to excessive accumulation of the lipofuscin in the RPE cells.⁴ In the macula, an abnormal ring of increased autofluorescence surrounding the fovea is present in 59% of patients with RP, representing an area of lipofuscin accumulation around a preserved subfoveal RPE.⁵ Although it is a nonspecific manifestation, the ring of increased autofluorescence in the perifoveal area has been suggested as an indicator of disease severity evaluated by pattern electroretinography, multifocal electroretinography, and visual field testing.⁶⁻⁹ The results of these tests reveal that the retinal function inside the ring is relatively more preserved than outside the ring.⁶⁻⁹ However, the structural basis of these high-density autofluorescence rings in patients with RP remains uncertain.

The recently developed noninvasive imaging technology, spectral-domain optical coherence tomography (OCT), allows for in vivo assessment of the retinal layers. In patients with RP, this technology can demonstrate thinning of the neurosensory retina and loss of the inner/outer segment (IS/OS) junction of the photoreceptors extrafoveally.¹⁰ In the center of the foveal region, the IS/OS junction is intact.¹⁰ These findings can be observed even with the earlier technology, the Stratus OCT (Carl Zeiss Meditec Inc., Dublin, CA),^{5,10} in which a “third hyperreflective band” was reported to be present along the length of the hyperautofluorescent ring and was absent outside its borders.⁵ This third band corresponds to the IS/OS junction of photoreceptor cilia, and to date, it is uncertain if the absence of the third band outside the ring corresponds to the absence of photoreceptors, the absence of outer segments, or disorganization of the IS/OS junction.⁵ Although the hyperautofluorescent ring may have diagnostic and prognostic implications, to our knowledge, there is no report on the integrity of the retinal layers underlying the ring in the literature. The purpose of this study is to analyze the underlying retinal layers by high-resolution spectral-domain OCT inside, across, and outside the hyperautofluorescent ring in patients with RP.

Methods

Subjects

This prospective cross-sectional study included 24 eyes of 13 patients with a clinical diagnosis of RP and with different diameters of hyperautofluorescent rings on fundus autofluorescence (FAF) (Figure 1). Twenty-four eyes of 13 control subjects with normal vision were also included. The patients' ages ranged from 13 years to 67 years and from 30 years to 65 years in the control subjects. The control subjects had visual acuities of 20/20 or better and normal eye examinations. The clinical diagnosis of RP was made by retinal specialists and was confirmed with full-field scotopic and photopic electroretinographies performed according to the International Society for Clinical Electrophysiology of Vision standards. All eyes in the study had best-corrected visual acuity of 20/20 (logMar = 0) and clear media to allow OCT and FAF imaging. Eyes were excluded if they had a refractive error $>\pm 6.0$ diopters spherical or ± 2.0 diopters cylindrical, evidence of cystoid macular edema, an epiretinal membrane, and evidence or a history of other ocular diseases (e.g., glaucoma, diabetes). Patients were enrolled with the approval of the Institutional Review Board protocol AAAB6560 at Columbia

University, and all research procedures were performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects before their enrollment.

Fundus Autofluorescence

Fundus autofluorescence imaging was performed with a confocal scanning laser ophthalmoscope (OCT-SLO Spectralis, Heidelberg Retina Angiograph 2, Heidelberg Engineering, Dossenheim, Germany) after pupil dilation with topical 0.5% tropicamide and 2.5% phenylephrine. Fundus autofluorescence imaging was performed using a 30° field of view at a resolution of $1,536 \times 1,536$ pixels. An optically pumped solid-state laser (488 nm) was used for excitation and a 495-nm barrier filter was used to modulate the blue argon excitation light. Standard procedure was followed for the acquisition of FAF images, including focus of the retinal image in the infrared reflection mode at 820 nm, sensitivity adjustment at 488 nm, and acquisition of 9 single 30° $\times$ 30° FAF images encompassing the entire macular area with at least a portion of the optic disk. The nine single images were computationally averaged to produce a single frame with improved signal-to-noise ratio.

The external and internal boundaries of the hyperautofluorescent ring were defined as the visible limits seen on FAF (Figure 2). The horizontal and vertical diameters of the hyperautofluorescent ring were measured for each hyperautofluorescent ring. The greatest linear dimension was defined as the horizontal diameter of the outer boundary. All the measurements were done using the 1,000- μ m caliper available on the FAF software.

Spectral-Domain Optical Coherence Tomography

Spectral-domain OCT was performed with the OCT-SLO Spectralis, Heidelberg Retina Angiograph 2 (Heidelberg Engineering) on 8 eyes. This equipment allows for simultaneous OCT scans and FAF imaging and subsequent image superimposition. Optical coherence tomography imaging was acquired by a broadband 870-nm superluminescent diode that scanned the retina at 40,000 A-scans per second with an optical depth resolution of 7 μ m. The standard protocol included 25 OCT scans averaged to reduce speckle noise by a factor of 5. The scans included at least one 9-mm horizontal line scan through the fovea.

Spectral-domain OCT images were also obtained on 16 eyes using the Cirrus Spectral Domain OCT (Carl Zeiss Meditec Inc, Dublin, CA). The acquisition protocol consisted of a 5-line raster scan and a macular cube 512 $\times$ 128 scan pattern in which a 6 mm $\times$ 6-mm region of the retina was scanned (a total of 65,536 sampled points) within a scan time of 2.4 seconds. After image acquisition, those with a signal strength ≤ 8 were excluded. Horizontal line scans through the center of the foveal region were repeated three times and images were registered and averaged. To compare FAF and Cirrus OCT scans, the 30° FAF images were overlaid onto red-free OCT images after they were appropriately scaled using an image registration software program created in MatLab (The MathWorks, Inc., Novi, MI).

The integrity of the underlying retinal layers, i.e., the outer nuclear layer (ONL), the external limiting membrane (ELM), the photoreceptor IS/OS segment junction, and the RPE complex, was analyzed inside, across, and outside the ring of increased autofluorescence. The thicknesses of the total retina (TR) and the photoreceptor, RPE complex layers (REC+) were measured across, outside, and inside the ring using Adobe Photoshop (Adobe Systems Incorporated, New York, NY) (Figure 3). The REC+ thickness included the outer plexiform layer, photoreceptor layer, RPE, and Bruch membrane. Inside the ring, all measurements were done from the foveal center. The locations at which thickness measurements were made varied across and outside the ring and were dependent on the diameter of the hyperautofluorescent ring. Thickness values were compared with those obtained from 24 normal control eyes. All

measurements were analyzed by two independent graders in the Department of Ophthalmology at Columbia University and in case of discrepancies, a third grader was asked to adjudicate.

Results

The clinical characteristics of the 13 patients are summarized in Table 1. There were 7 women and 6 men whose ages ranged from 13 years to 67 years. Snellen best-corrected visual acuity for all tested eyes was 20/20. Ten of the patients had autosomal-dominant or autosomal-recessive RP, two had Usher syndrome, and one patient had Bardet-Biedl syndrome. Full-field scotopic electroretinography responses were severely diminished and photopic responses were decreased in amplitude and delayed in implicit time for all 24 eyes compared with normal control values.

The hyperautofluorescent rings detectable by FAF were not clinically visible on funduscopy, and none of the studied patients showed a bull's eye-appearing macular lesion. All 24 eyes had an abnormal hyperautofluorescent perifoveal ring. External and internal boundaries of the hyperautofluorescent perifoveal ring were observed in all the eyes on FAF and all them had an ovoid shape oriented horizontally. The greatest linear dimension represented the horizontal diameter of the external boundaries and it ranged from 2,369 μm to 7,405 μm . The vertical diameter ranged from 1,776 μm to 6,720 μm . The horizontal diameter of the inner boundary ranged from 1,639 μm to 6,860 μm , and the vertical diameter ranged from 1,214 μm to 5,576 μm . The dimensions of the perifoveal rings are listed in Table 2.

The retinal layers observed on the spectral-domain OCT images associated with the hyperautofluorescent ring were analyzed. Inside the ring, an area of normal foveal autofluorescence and structure with intact ELM, IS/OS junction, and ONL was observed in all 24 eyes. At the transitional zone of the ring, breakdown of IS/OS junction was observed, the ONL was decreased in thickness in a centrifugal direction, and the ELM was barely visible. Outside the hyperautofluorescent ring, no IS/OS junction, ELM, or ONL was detected (Figure 4).

In all 24 eyes analyzed, the TR and REC+ thicknesses were decreased across and outside the ring in comparison to measurements obtained on 24 normal control eyes. The amount of retinal thickness decrease was greater outside than across the transitional zone of the hyperautofluorescent ring. Inside the ring, the foveal thickness was similar to that of the control eyes (Tables 2 and 3).

Discussion

On macular FAF, a ring of high density is often observed in patients with RP. The hyperautofluorescent rings are reported to constrict progressively in some patients, and this constriction may be detectable after a relatively short follow-up period.⁷ Constriction of the ring mirrors the progressive visual field loss.⁶ The prognosis for retention of central vision may be better in patients with large or slowly changing rings. The absence of detectable autofluorescent ring constriction in other patients with normal visual acuity may reflect milder or less progressive macular involvement. Robson et al^{11,12} stated that hyperautofluorescent areas represent a transition between abnormal paracentral and normal central cone system function.

Our OCT findings reflect that there are structural alterations in the retina, which correlate with the hyperautofluorescent ring. Specifically, we evaluated the integrity of the outer retinal layers inside, across, and outside the ring of hyperautofluorescence. In addition, we measured the thicknesses of the TR and REC+. Inside the ring, the TR and REC+ thickness values were within the 95% confidence interval of normal control values. These findings are consistent

with reports that the rings correlate with measures of macular function by pattern electroretinography and fine matrix mapping. It has been suggested that the rings may be of prognostic value in predicting retention of good central vision.⁶

The size of the ring seems to be of value in prognosticating the rate of visual loss in patients with RP. Recent publications studying the size of the ring and its relationship to multifocal electroretinography and pattern electroretinography P50 response amplitudes demonstrated a positive correlation among them, i.e., retinal function was preserved inside the ring.^{6,8,9,12,13}

Transversing the ring at the hyperautofluorescent transition zone, we observed IS/OS junction disorganization, the ONL was thinner, and the ELM was barely detectable. The thickness values of the TR and REC+ were decreased compared with the normal control values. Outside the hyperautofluorescent ring, there was loss of the IS/OS junction, ELM, and ONL, and both TR and REC+ thickness values were markedly decreased. These results are consistent with previous studies demonstrating impaired visual function outside the hyperautofluorescent ring.^{6,8,9}

Early disorganization of the IS/OS boundary is a morphologic marker for photoreceptor damage in human^{13,14} and animal models.^{15,16} Loss of the IS/OS junctions at the transitional zone of the hyperautofluorescent ring suggests that the photoreceptor cilia, i.e., the IS/OS, possibly triggers subsequent development of apoptosis. Similar perifoveal rings are also seen in cone-rod dystrophy,¹⁷ X-linked retinoschisis,¹⁸ and Leber congenital amaurosis.¹⁹

The hyperautofluorescent ring represents an abnormal perifoveal accumulation of lipofuscin in the RPE as a result of an increased outer segment degeneration as a precursor of apoptosis. Because retinal degeneration in RP classically begins in the retinal periphery, the preserved visual fields of eyes with large rings are wider than those for eyes with smaller rings. The progressive constriction in diameter of the ring is a sign of disease progression in patients with RP. Our data support the hypothesis that photoreceptor degeneration initiates at the IS/OS junction long before the loss of the photoreceptors nuclei. Therefore, IS/OS junction disorganization might be an early indication of programmed cell death in RP.

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Fig. 1.
A–F, Fundus autofluorescence images showing variably sized perifoveal rings of hyperautofluorescence for Patients 1, 3, 5, 8, 9, and 11, respectively. Autofluorescence is normal inside the ring of surviving retina and abnormal outside the ring.

Fig. 2.

A, Right eye of Patient 8 showing a larger ring. This ring measured $7,405\ \mu\text{m}$ in the greatest linear dimension and $5,576\ \mu\text{m}$ in the vertical diameter. **B**, Left eye of Patient 1 with a small ring measuring $2,369\ \mu\text{m}$ in the great linear dimension, i.e., the horizontal diameter of the outer boundary of the hyperautofluorescent ring. The vertical diameter measured $1,214\ \mu\text{m}$.

Fig. 3.

A normal right eye showing layers that represent total retinal and REC+ thickness. RNFL, retinal nerve fiber layer; IPL, inner plexiform layer; RGC, retinal ganglion cell; INL, inner nuclear layer; OPL, outer plexiform layer.

Fig. 4.

A and B, The right eye of Patient 5. **C and D,** The right eye of Patient 6. **E and F,** The left eye of Patient 5. **G and H,** The right eye of Patient 1. All four images illustrate that across the ring, no IS/OS junction is observed, the ONL is decreased in thickness, and the ELM is barely visible. Outside the hyperautofluorescent ring, no IS/OS junction, ELM, or ONL is observed. Inside the ring, all retinal layers are observed and have normal thickness. Intact IS/OS junction seems to contribute to the formation of the hyperautofluorescent ring seen in patients with RP. *IS/OS junction.

Table 1
Clinical Characteristics of 13 Patients With RP and Hyperautofluorescent Rings

Patient No.	Diagnosis	Age (Years)	BCVA	
			OD	OS
1	Autosomal-dominant RP	49	20/20	20/80
2	Autosomal-dominant RP	46	20/20	20/20
3	Autosomal-recessive RP	13	20/20	20/20
4	Bardt-Biedel syndrome	16	20/20	20/20
5	Usher syndrome type II	39	20/20	20/20
6	Usher syndrome type II	22	20/20	20/20
7	Autosomal-recessive RP	15	20/20	20/20
8	Autosomal-recessive RP	67	20/20	20/20
9	Autosomal-recessive RP	55	20/20	20/20
10	Autosomal-recessive RP	21	20/20	20/20
11	Autosomal-dominant RP	47	20/20	20/20
12	Autosomal-recessive RP	29	20/20	20/20
13	Autosomal-recessive RP	34	20/20	20/20

BCVA, best-corrected visual acuity; OD, right eye; OS, left eye.

Table 2

Values of the Internal and External Boundaries Diameter of the Ring in the Vertical and Horizontal Axis

	Range of the Vertical Diameter of the Ring (Mean $\pm$ 1 SD)	Range of the Horizontal Diameter of the Ring (Mean $\pm$ 1 SD)
Internal boundaries	1214–5576 μm (2962 $\pm$ 1683)	1639–6860 μm (3654 $\pm$ 2038)
External boundaries	1776–6720 μm (4043 $\pm$ 1834)	2369–7405 μm (4784 $\pm$ 1909)

SD, standard deviation.

Table 3

TR Thickness and Thickness of REC+ Layers in the Center of the Fovea, Between the Inner and Outer Boundaries of the Ring (Across the Ring), and Temporal to the Outer Boundary (Outside the Ring) for 13 Patients With RP

TR (μm)	Fovea Patient (95% CI)	Across the Ring (95% CI) Patient	Outside Patient (95% CI)	REC+ (μm)	Across the Ring (95% CI) Patient	Outside Patient (95% CI)
1	OD: 247.4 (188.9–284.8) OS: 282.3 (188.9–284.8)	OD: 304.7 (327.4–362.3) OS: 274.9 (303.9–407.3)	OD: 301.2 (345.2–360.8) OS: 238.9 (290.6–396.3)	1	OD: 151.3 (178.3–210.4) OS: 104.2 (155.9–187.1)	OD: 155.8 (164.5–203.4) OS: 73.6 (144.8–190.7)
2	OS: 266.7 (191.2–285.9) OD: 263.6 (188.9–284.8)	OS: 306.5 (317.7–403.3) OD: 236 (292.2–395.8)	OS: 245.6 (293.5–399.6) OD: 215.1 (282.2–381.9)	2	OS: 144.1 (157.8–189.1) OD: 97.4 (131.5–190.6)	OS: 89.7 (148.5–193.2) OD: 94.8 (130.4–181.3)
3	OS: 266.7 (191.2–285.9) OD: 198.2 (188.9–284.8)	OS: 242.7 (293.2–392.5) OD: 271.5 (317.1–350.2)	OS: 220.6 (283.8–387.3) OD: 265.8 (344.3–360.5)	3	OS: 123.6 (133.9–194.7) OD: 120.3 (180.7–217.4)	OS: 113.3 (133.2–185.3) OD: 90.5 (168.2–203.5)
4	OS: 193.6 (191.2–285.9) OD: 264.7 (188.9–284.8)	OS: 303.4 (317.9–348.9) OD: 245 (292.2–395.8)	OS: 296.8 (341.8–360.9) OD: 213.5 (282.2–381.9)	4	OS: 126.4 (185.1–218.3) OD: 99 (131.5–190.6)	OS: 93.2 (167.7–204.9) OD: 93.2 (130.4–181.3)
5	OS: 267.9 (191.2–285.9) OD: 197.5 (188.9–284.8)	OS: 243.4 (293.2–392.5) OD: 272.6 (317.1–350.2)	OS: 222.8 (283.8–387.3) OD: 266.7 (344.3–360.5)	5	OS: 121.4 (133.9–194.7) OD: 123.4 (180.7–217.4)	OS: 114.2 (133.2–185.3) OD: 93.8 (168.2–203.5)
6	OS: 200.7 (191.2–285.9) OD: 196.8 (188.9–284.8)	OS: 287.6 (317.9–348.9) OD: 274.8 (292.2–395.8)	OS: 267.9 (341.8–360.9) OD: 264.9 (282.2–381.9)	6	OS: 125.7 (185.1–218.3) OD: 123.7 (131.5–190.6)	OS: 94.9 (167.7–204.9) OD: 94.9 (130.4–181.3)
7	OD: 199.5 (188.9–284.8) OS: 198.8 (191.2–285.9)	OD: 275.2 (317.1–350.2) OS: 274.9 (317.9–348.9)	OD: 266.4 (344.3–360.5) OS: 265.1 (341.8–360.9)	7	OD: 121.5 (180.7–217.4) OS: 127.2 (185.1–218.3)	OD: 92.6 (168.2–203.5) OS: 95.9 (167.7–204.9)
8	OD: 197.7 (188.9–284.8) OS: 196.3 (191.2–285.9)	OD: 277.5 (318.3–348.9) OS: 272.9 (316.1–342.6)	OD: 267.9 (341.3–358.2) OS: 266.3 (342.6–361.4)	8	OD: 122.3 (186.7–215.9) OS: 129.4 (185.7–214.8)	OD: 97.9 (166.6–202.3) OS: 94.6 (168.1–203.7)
9	OD: 194.3 (188.9–284.8) OS: 197.1 (191.2–285.9)	OD: 278.3 (318.3–348.9) OS: 273.6 (316.1–342.6)	OD: 268.5 (341.3–358.2) OS: 265.7 (342.6–361.4)	9	OD: 125.8 (186.7–215.9) OS: 126.3 (185.7–214.8)	OD: 91.3 (166.6–202.3) OS: 93.5 (168.1–203.7)
10	OD: 193.9 (188.9–284.8) OS: 196.3 (191.2–285.9)	OD: 271.8 (318.3–348.9) OS: 270.4 (316.1–342.6)	OD: 263.1 (341.3–358.2) OS: 268.4 (342.6–361.4)	10	OD: 127.4 (186.7–215.9) OS: 108.4 (185.7–214.8)	OD: 94.8 (166.6–202.3) OS: 93.6 (168.1–203.7)
11	OD: 201.5 (188.9–284.8) OS: 203.7 (191.2–285.9)	OD: 273.7 (317.1–350.2) OS: 275.2 (317.9–348.9)	OD: 265.9 (344.3–360.5) OS: 262.4 (341.8–360.9)	11	OD: 103.9 (131.5–190.6) OS: 102.4 (133.9–194.7)	OD: 101.5 (130.4–181.3) OS: 99.7 (133.2–185.3)
12	OD: 199.6 (188.9–284.8) OS: 197.2 (191.2–285.9)	OD: 271.9 (317.1–350.2) OS: 275.8 (317.9–348.9)	OD: 270.4 (344.3–360.5) OS: 269.5 (341.8–360.9)	12	OD: 109.3 (133.9–194.7) OS: 101.2 (133.9–194.7)	OD: 100.4 (130.4–181.3) OS: 107.9 (133.2–185.3)

The confidence interval was calculated from 24 normal eyes. 95% CI, 95% confidence interval; OD, right eye; OS, left eye.