Correlation between Macular Morphology and Sensitivity in Patients with Retinitis Pigmentosa and Hyperautofluorescent Ring

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PURPOSE. To assess the correlation between retinal morphology and function in patients with retinitis pigmentosa (RP) using spectral domain optical coherence tomography (SD-OCT), fundus autofluorescence imaging (FAF), and fundus-related perimetry and to use coregistration of data sets to achieve high-resolution structure-function correlation of human macula.

METHODS. Twelve patients with RP and hyperautofluorescent parafoveal ring in FAF imaging were tested. Ophthalmological examination, static and kinetic fundus-related perimetry, and SD-OCT were performed. Custom software allowed coregistration of fundus-related perimetry, SD-OCT, and FAF data sets.

RESULTS. A high correlation between retinal sensitivity and outer retinal thickness was observed ($r = 0.72, P < 0.0001$). The median retinal sensitivity over the central circular area of normal autofluorescence was significantly higher when compared with the area over the surrounding hyperautofluorescent ring and to the area outside the ring ($H = 34.2, P < 0.0001$). The outer retina at the site where kinetic stimuli were perceived was better preserved and had higher retinal thickness, corresponding to higher sensitivity ($H = 289, P < 0.0001$). The site of the hyperautofluorescent ring correlated in SD-OCT scans with a zone of impaired integrity of the photoreceptor layer ($r = 0.67, P = 0.0005$).

CONCLUSIONS. Retinal sensitivity to static and kinetic stimuli correlates better with outer than with overall retinal thickness. The hyperautofluorescent ring in FAF represents a transition zone from relatively well-preserved to abnormal retinal morphology and function, rendering FAF imaging a clinically significant tool for assessing the severity and progression of dysfunction in RP patients. Accurate coregistration of different modalities drastically increases the power of structure-function correlation studies and allows consistent associations to be drawn. (Invest Ophthalmol Vis Sci. 2012;53:47–52) DOI: 10.1167/iovs.11-8048

RP

etinitis pigmentosa (RP [MIM 268000]) denotes a group of genetically determined retinal dystrophies exhibiting immense clinical and genetic heterogeneity and is characterized by night blindness and progressive visual field loss. Forms of this condition differ in severity, natural history, and mode of inheritance.

Ophthalmic imaging and perimetry testing have long played an important role in the documentation and diagnosis of conditions such as RP. In the past decade, noninvasive retinal imaging technologies such as optical coherence tomography have significantly increased our understanding of structural changes in retinal disease; specifically, spectral domain optical coherence tomography (SD-OCT) has enabled high-resolution, 3-dimensional (3D), in vivo visualization of retinal morphology.1 Additionally, imaging techniques such as fundus autofluorescence (FAF) have allowed characterization of the spatial distribution and intensity of lipofuscin-derived autofluorescence. By marking the accumulation of lipofuscin granules in retinal pigment epithelium (RPE), FAF provides a molecular marker of RPE health.2 Fundus-controlled perimetry allows functional assessment of the central retina with high spatial resolution. Along with FAF and SD-OCT, they provide a basic toolset with which to analyze the structure, molecular composition, and function of the central retina in vivo.

Understanding how function, structure, and molecular footprint are interconnected enables accurate assessment of disease progression and is pivotal for clinical trials.3 Correlation of different modalities enables precise alignment of data sets, thus allowing for the statistical analysis of relationship and the study of correlation. Both custom-made image registration software overlaying different data sets4–7 and instruments automatically combining structural and functional modalities (OPKO SLO/SD-OCT microperimeter; OPKO, Miami, FL)8 have been used for this purpose.

Previous studies have reported abnormal FAF in the form of a parafoveal ring of increased signal in RP patients;9–12 this distinctive autofluorescence phenotype has been reported in more than 50% of subjects with RP.13 Similar ring or ringlike structures have been described in other inherited retinal dystrophies.14,15 Although it is a nonspecific finding, the ring seems to be of prognostic value and is useful in assessing the degree of macular dysfunction in patients with RP.9,11,14,16,17 When SD-OCT was performed, loss of the hyperreflective band corresponding to the photoreceptor inner and outer segment (IS/OS) junction was observed at the transitional zone of the hyperautofluorescent ring.17–20

The structural and functional significance of the hyperautofluorescent ring as well as its prognostic and monitoring value have been well established.9–12,14,17,21–25 However, there is still a need for a more robust correlation between structural abnormalities and functional deficits. In the present study, we have evaluated 12 patients with RP who had parafoveal ring of hyperautofluorescence. The purpose of this study was to assess the correlation of visual function measured by both static and kinetic fundus-related perimetry with structural changes in the

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inner and outer retina evaluated with SD-OCT and with molecular changes in the RPE visualized with FAF.

**Subjects and Methods**

**Study Subjects**

Twelve patients from 11 families with clinically and electrophysiologically confirmed diagnoses of RP, hyperautofluorescent ring on FAF, retained central vision, and stable fixation were included in this cross-sectional study. Six patients had nonsyndromic autosomal recessive RP; three had autosomal dominant RP and three had Usher syndrome type 2. Median age was 40 years (range, 23–72 years). Median best-corrected visual acuity was 1.0 (range, 0.3–1.0). The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and adhered to the tenets of the Declaration of Helsinki.

FAF imaging (Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany) and SD-OCT (3D OCT-1000; Topcon, Tokyo, Japan) were performed in all subjects. Our SD-OCT protocol included a volume scan covering a 6 mm (horizontal) × 6 mm (vertical) × 1.7 mm (axial) block of the macular region, centered on the fovea. Fundus-controlled static and kinetic perimetry (MP1 Microperimeter; Nidek Technologies, Padova, Italy) were performed in 8 and 11 patients, respectively. For static perimetry (Humphrey 10–2), we tested 56 retinal locations in the central 20°, with 2° resolution at threshold sensitivities from 0 to 20 dB; the test spot size was Goldmann III. For kinetic perimetry (Goldmann III), stimuli with five luminance levels (0, 4, 8, 12, 16 dB) were used. The stimuli were moving centripetally from 20° to the starting point in the center of the macula with an automated algorithm in eight directions and a velocity of 2.4°/s. All patients had previously undergone static and kinetic visual field testing and were familiar with the testing procedure.

**Coregistration of Functional Testing, Autofluorescence Imaging, and Retinal Thickness Profiles**

A custom-made software tool, MultiModalMapper 1.1, was used to accurately align FAF, MP1, and SD-OCT data sets using anatomic landmarks. The MultiModalMapper 1.1 software is not a commercial product. The software was developed as WPF application (Microsoft Windows Presentation Foundation; Microsoft, Redmond, WA) using the Microsoft NET framework 3.5 sp1. To map FAF and MP1 on SD-OCT data, we manually selected at least three landmarks on related fundus images. The result of the coregistration process was visualized immediately as an overlaid image to allow for adjustment by moving or adding additional landmarks. After coregistration and alignment, the SD-OCT data set was rendered as a 3D model, with the FAF image and the MP1 color-coded data layers displayed as textured planes. Although a number of automatic segmentation algorithms have been developed, their accuracy in pathologic eyes remains to be demonstrated; in this study, manual segmentation by an expert grader was performed. Markers for the inner limiting membrane, the inner border of the outer plexiform layer (OPL), and the outer border of RPE were manually positioned in MultiModalMapper with an accuracy of 4 μm or better (depending on the chosen zoom level during segmentation). Subsequently, these markers were used to define borderslines and create thickness maps for total (from the vitreoretinal interface to the outer border of the RPE), inner (from the vitreoretinal interface to the inner border of the OPL), and outer (from the inner border of the OPL to the outer border of the RPE, including the axons of the photoreceptors) retina. Total, inner, and outer retinal thicknesses were determined for each MP1 data point and for each of the three distinct FAF regions (the hyperautofluorescent ring, the area enclosed by the hyperautofluorescent ring, and the area outside the hyperautofluorescent ring). On the site of kinetic perimetry data points, qualitative analysis of the structural integrity of the IS/OS junction line was performed. The IS/OS junction line was characterized as absent (complete disappearance of the IS/OS junction line), disrupted (disorganization of the IS/OS junction line), or preserved (intact IS/OS junction line).

**Statistical Analysis**

Statistical analysis was performed with statistical software (Prism 4.0; GraphPad Software, San Diego, CA). Data are reported as median with corresponding interquartile ranges. Spearman rank correlation coefficient (ρ) was used to measure the association between static perimetry values and retinal thickness; a nonparametric (distribution-free) test was chosen because the calculated parameters were not distributed normally. We plotted retinal thickness as a function of retinal sensitivity in linear units because it was previously shown that a simple linear model accurately relates SD-OCT to retinal sensitivity parameters. For kinetic perimetry, results were ordered into five groups (0, 4, 8, 12, and 16 dB). Inner, outer, and total retinal thicknesses were evaluated at each data point, and a nonparametric test (Kruskal-Wallis one-way ANOVA) was used to compare the five independent groups of sampled data. Statistic H was compared with a χ² distribution with 4 df, and P was computed. Based on fundus autofluorescence imaging, the retina was split into three groups: the area within the ring of high density, the area over the ring, and the area peripheral to the ring. Kruskal-Wallis ANOVA was used to compare retinal thickness or sensitivity in those three groups; 2 df was used to compute P. The alternative hypothesis for Kruskal-Wallis states that at least one median is different from the rest. Therefore, when the result was significant, Dunn posttest (multiple, pairwise, stepdown comparisons) was used to identify those groups that caused the Kruskal-Wallis test to reject the null hypothesis. P < 0.05 was considered significant.

**Results**

**Correlation between Fundus-Related Perimetry and SD-OCT**

Thickness of the segmented layers was assessed at the spots corresponding to the static perimetry data points. Both total and outer retinal point thickness correlated with retinal sensitivity, with a positive correlation coefficient indicating that when thickness increases, sensitivity increases (ρ = 0.54 and 0.72; P < 0.0001 and P < 0.0001). Correlation for outer retinal thickness and sensitivity was the highest in magnitude; these data are presented in Figure 1.

Structural integrity and thickness of the outer retina were evaluated at the kinetic perimetry data points. Perimetry results were ordered into five groups (0, 4, 8, 12, and 16 dB), and median thickness across groups was compared (Fig. 1). There was strong evidence that outer retinal thickness was different in at least one sensitivity group (H = 288.6; 4 df, P < 0.0001). Multiple comparisons of each pair of groups gave P < 0.05 for all (Dunn posttest). Qualitative structural integrity of the IS/OS junction line for kinetic MP1 data points is presented in Figure 2. There was no statistically significant difference in retinal thickness and presence of IS/OS on the site of recognition of 0 dB between kinetic and static perimetry (P = 0.574, Mann-Whitney U test).

**Comparison between FAF and SD-OCT**

The thickness profile was assessed inside, over, and outside the hyperautofluorescent ring in each eye. H values were 17.0 (P = 0.0002), 61.5 (P < 0.0001), and 41.5 (P < 0.0001) for inner, outer, and total retinal thickness, respectively, rejecting the null hypotheses (no difference among groups). Multiple pairwise comparisons between groups to locate the source of significance found critically different outer and total retinal thickness between the three areas (Dunn posttests after two Kruskal-Wallis tests for outer and total retina). For the inner
retinal thickness, the pairwise comparisons revealed differences between the area inside and over as well as inside and outside the ring but not between the area over and outside the ring (Dunn posttests). Results are summarized in Figure 3. A high correlation between the diameter of the inner border of the hyperautofluorescent ring and the IS/OS junction line on the OCT was observed ($r = 0.67$, $P = 0.0003$; Fig. 4).

Comparison between FAF and Fundus-Related Perimetry

Static perimetry data points were grouped into those inside, those over, and those outside the ring of high density (Fig. 3). Sensitivity values were collected for each spot. The results of a Kruskal-Wallis test were significant ($H = 34.2, 2$ df, $P < 0.0001$), and the mean ranks of retinal sensitivity were significantly different among the three areas (Dunn posttest).

DISCUSSION

SD-OCT, static, and kinetic fundus-related perimetry were performed in patients with RP and parafoveal ring of increased signal on FAF imaging. Custom-made software was used to coregister the three different modalities, representing retinotopic maps of structure, molecular composition, and function of the central retina. To more accurately assess morphologic alterations and their functional consequences, manual segmentation was performed. We have shown that retinal sensitivity correlates better with outer as opposed to total retinal thickness. Additionally, the inner retina was relatively preserved, which is critical for novel therapeutic approaches such as retinal prostheses.

The ring of hyperautofluorescence can encircle either preserved (RP) or diseased retina (cone dystrophies, maculopathies), representing an accumulation of lipofuscin in a transition zone. Understanding this transition zone has implications for treatment strategies and helps delineate the nature of disease progression. In our cohort of RP patients, SD-OCT imaging revealed the preserved IS/OS junction line inside the ring, confirming the results of previous studies. At the inner border of the hyperautofluorescent ring, outer retinal thickness sharply decreased; this was not the case with inner retinal thickness, which was relatively preserved. On static perimetry, inside the hyperautofluorescent ring, retinal sensitivity was relatively preserved. On the
site of the ring sensitivity decreased, and outside the ring an absolute scotoma was observed. In agreement with a recent study by Greenstein et al., statistically significant differences in retinal sensitivity among these three groups were observed. These results are in accordance with the notion that the hyperautofluorescent ring represents the transition zone between functional and dysfunctional retina. Notably, recent evidence suggests that structural and functional changes may also occur inside the ring.

Kinetic perimetry is superior to static perimetry for evaluating peripheral visual fields and delineating scotomata but is less sensitive in detecting small central visual field defects. Fundus-related kinetic perimetry enables observation of fixation and allows precise delineation of scotomata at the posterior pole. In this study, we have evaluated outer retinal structural integrity and thickness at specific kinetic perimetry data points. Low-intensity (16 dB) stimuli were seen only where the IS/OS line was preserved, whereas high-intensity (0 dB) stimuli could be seen despite complete loss of the IS/OS line. Similar results have been reported in a study in which Goldmann perimetry (in which patient fixation cannot be carefully monitored) and time-domain OCT were used; this was attributed to poor patient compliance and low OCT resolution. Reaction time is expected to induce displacement of the isopter toward the direction of the stimulus movement. In RP patients, reaction time is prolonged and is estimated to be 702 ms. The velocity of the stimulus movement in this study was 2.4°/s; hence, a shift of 1.68° would be expected. This would mean that the signal was recognized at 1.68° or approximately 0.4 mm before the location at which the retinal structure was evaluated. Therefore, we feel that the recognition of kinetic perimetry stimuli despite the IS/OS line loss is not artifactual. This is in agreement with results of a previous study using static perimetry and showing that the IS/OS line had essentially disappeared when sensitivity was reduced by 10 dB or more and highlights that increased photoreceptor density is critical for the recognition of low-intensity but not of high-intensity perimetry stimuli. Further studies with cellular resolution in vivo retinal imaging using adaptive optics will allow visualization of residual outer segment material (below resolution

FIGURE 3. Static fundus-related perimetry (A), outer (B), and inner (C) retinal thickness maps superimposed on a fundus autofluorescence image. Red lines: inner border of the hyperautofluorescent ring; blue lines: outer border of the hyperautofluorescent ring. Median retinal sensitivity (D), outer (E), and inner (F) retinal thickness with interquartile range inside, over, and outside the hyperautofluorescent ring is shown.

FIGURE 4. Comparison between the diameter of the inner border of the hyperautofluorescent ring and the length of inner and outer segment junction line on OCT ($\rho = 0.67, P = 0.0003$).
threshold of standard SD-OCT) and will facilitate more accurate assessment.

By performing both static and kinetic fundus-related perimetry, we were able to demonstrate reduced sensitivity to a static stimulus as opposed to the identical kinetic stimulus at certain field locations of some patients. This phenomenon is known as statokinetic dissociation and was first reported in 1917 by George Riddoch in patients with occipital lobe lesions. Since then it was described in many other conditions, including RP. Wood et al. showed that the kinetic nature of the stimulus is more effective in detecting small degrees of residual field in RP. In this study we compared retinal structure on the site of recognition of 0 dB between kinetic and static perimetry, and no statistically significant difference in either retinal thickness or presence of IS/OS was identified. Therefore, it can be speculated that the dissociation of static and kinetic perimetry is unlikely to be due to outer retinal contribution.

Our study has some limitations that must be addressed. Both retinal sensitivity and thickness vary between individuals and with eccentricity from the foveal center. Nevertheless, a large data set of normative MP1 data (n = 190 eyes) has revealed only a small difference in retinal sensitivity between peripheral and central areas (18.7 ± 1.2 dB vs. 19.4 ± 0.8 dB). Additionally, in our protocol, most MP1 data points (54/56) fell within the inner and outer ETDRS subfields, where observed variation in retinal thickness is small. Therefore, a significant confounding effect is not expected. Additionally, accurately measuring the photoreceptor nuclei thickness remains a challenge, and higher resolution SD-OCT will provide further insight.

In patients with RP, a hyperautofluorescent ring on FAF imaging separates areas with different structural and functional characteristics. We have used a variety of methods to show that these differences are statistically significant, highlighting the clinical usefulness of FAF in RP. The importance of structure-function correlation studies is difficult to understated. The segmentation of OCT volume scans, the combination of static and kinetic perimetric methods, and the accurate coregistration of different modalities drastically increases the power of these studies and allows consistent associations to be drawn.

**References**


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