

# Photopic and Scotopic Fine Matrix Mapping of Retinal Areas of Increased Fundus Autofluorescence in Patients with Age-Related Maculopathy

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**PURPOSE.** To investigate photopic and scotopic sensitivity of retinal areas that show increased fundus autofluorescence (FAF) in patients with age-related maculopathy (ARM).

**METHODS.** FAF was imaged with a modified confocal scanning laser ophthalmoscope (cSLO). Fine matrix mapping (FMM) was performed with a modified field analyzer. Photopic and scotopic thresholds were obtained at 100 locations on a  $9^\circ \times 9^\circ$  matrix with  $1^\circ$  spacing, centered on a macular area of increased FAF. Inclusion criteria included ARM fundus changes, areas of increased FAF, central and stable fixation, and visual acuity of 20/40 or better.

**RESULTS.** FAF images were reviewed in 436 patients with age-related maculopathy (ARM), of whom 38 met the inclusion criteria. FMM was performed in seven eyes of seven patients. Areas of increased FAF in patients with late ARM (choroidal neovascularization or geographic atrophy) showed normal or only mildly abnormal photopic, but severely reduced scotopic, sensitivity. The central area of increased FAF corresponding to a large foveal druse in a patient with ARM showed moderately reduced photopic and severely reduced scotopic sensitivity. In the other patients with ARM with drusen, areas of increased FAF showed normal or near-normal photopic sensitivity, but moderately reduced scotopic sensitivity.

**CONCLUSIONS.** In retinal areas of increased FAF in patients with ARM, scotopic sensitivity loss considerably exceeded photopic sensitivity loss. This finding is in line with histologic data that have demonstrated a preferential loss of rods in ARM, but does not explain the magnitude of sensitivity loss. The study shows that increased FAF in ARM has a functional correlate. (*Invest Ophthalmol Vis Sci.* 2004;45:574-583) DOI:10.1167/iovs.03-0495

Age-related maculopathy (ARM) is a degenerative disorder of the central retina (the macula) in persons older than 50 years. The early stages of ARM are characterized by soft drusen

(larger than  $63 \mu\text{m}$ ), areas of increased pigment or hyperpigmentation (in the outer retina or choroid), and/or areas of depigmentation or hypopigmentation of the retinal pigment epithelium (RPE). The late stages of ARM, age-related macular degeneration (AMD), include geographic atrophy (GA) and choroidal neovascularization (CNV).<sup>1</sup> Late ARM is the leading cause of severe, irreversible central visual loss and legal blindness among older adults in Europe<sup>2,3</sup> and the United States<sup>4,5</sup> and in other predominantly white populations.<sup>6,7</sup> The pathogenesis of ARM is poorly understood, but it is agreed that its prominent clinical and histopathological features involve Bruch's membrane, the RPE, and the photoreceptors.<sup>8,9</sup>

The ability to image fundus autofluorescence (FAF) allows age changes in the RPE to be documented clinically, at least with respect to acquisition of FAF derived from lipofuscin.<sup>10-15</sup> Images of the distribution of FAF, obtained using a confocal scanning laser ophthalmoscope (cSLO), were first described by von Ruckmann et al.<sup>13</sup> Areas of increased FAF are a frequent feature of ARM.<sup>14,16</sup> The lack of obvious correspondence between the distribution of FAF and drusen<sup>14,16</sup> and thickening of Bruch's membrane<sup>17</sup> appears to indicate that FAF and changes in Bruch's membrane are two independent measures of aging and ARM in the retina. However, the functional implications of increased FAF in ARM are still not clear.

The RPE, Bruch's membrane, and the choroid are vitally important for the health of photoreceptors. It is the dysfunction and death of photoreceptors that accounts for the visual loss associated with ARM. Therefore, photoreceptor function, assessed *in vivo*, is the most direct bioassay of the significance of changes in the RPE-Bruch's membrane complex. Recently, it has been shown that in ARM the involvement of the rod system precedes that of the cone system (for a review see Refs. 18-20).

To investigate the functional implications of increased FAF in ARM and to determine the involvement of both the cone and the rod system we performed photopic and scotopic fine matrix mapping (FMM) in patients with ARM that exhibited areas of increased macular FAF.

## MATERIALS AND METHODS

Patients were selected for the study from a large ARM database of clinical data, fundus images, and FAF images. Inclusion criteria for the study included ARM fundus changes according to the International ARM Epidemiologic Study Group,<sup>1</sup> areas of increased FAF, and a visual acuity of 20/40 or better. Patients with other changes (e.g., diabetic retinopathy) were excluded. Because significant lens opacities may impair the quality of FAF images,<sup>21</sup> patients with lens opacities that exceeded mild nuclear sclerosis on slit lamp examination were also excluded. This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Moorfields Hospital ethics committee.

The protocol of the study was as follows: (1) best corrected visual acuity with Early Treatment Diabetic Retinopathy Study (ETDRS) charts, (2) standard visual field examination (Humphrey Field Analyzer;

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Carl Zeiss Meditec, Dublin, CA) using the 30-2 program (data not shown), (3) photopic FMM, (4) pupil dilatation with 1.0% tropicamide and 2.5% phenylephrine, (5) dark adaptation for 45 minutes, (6) dark-adapted 30-2 visual field examination as described elsewhere<sup>22</sup> (data not shown), (7) scotopic FMM, (8) FAF recording, and (9) determination of the fixation location and measurement of fixation stability. The patients were allowed to rest between procedures.

Depending on the location of the area of increased FAF, either FMM of the center of the macula or of the nasal superior, nasal inferior, temporal superior, or temporal inferior quadrant of the macula was obtained, each of which included the fovea at one of the corners of the FMM. The technique of FMM has been described before.<sup>22-28</sup> Briefly, in this technique a modified Humphrey field analyzer is used. For the photopic FMM measurements, standard Humphrey size III target white flashes were used on a standard Humphrey bowl illumination of 31.5 apostilbs. For scotopic FMM measurements, Humphrey size III target blue flashes were presented with the bowl illumination switched off under scotopic conditions. Four red-light-emitting diodes in a small-diamond configuration served as the fixation target. Accuracy of fixation was monitored by means of an infrared camera. To perform the FMM perimetry the coordinates of four interlaced  $5 \times 5$  grids at 25 locations (with a separation between adjacent points of  $2^\circ$ ) were entered in the "Custom Grid" feature of the Humphrey automated perimeter. Each grid is offset relative to the other grids by  $1^\circ$ , in the  $x$ ,  $y$ , or  $x$  and  $y$  axes. The data were converted into IBM format files that were merged using custom software to produce a single matrix with a separation between test locations of  $1^\circ$ . Thus, the FMM includes 100 test locations and subtends a visual angle of  $9^\circ \times 9^\circ$ . The FMM thresholds underwent spatial processing with a  $3 \times 3$  Gaussian (normal) filter. This technique, which has been used to filter conventional Humphrey 30-2 field data and FMMs and to improve repeatability further, has been described in detail.<sup>28,29</sup> The numerical matrix of the luminance sensitivity at each of the test locations was then used to generate a surface or contour plot showing the size and location of luminance sensitivity gradients across the grid (contour steps: 0.1 log unit). The luminance sensitivity contour plots were superimposed onto the FAF images with custom image-analysis software. Accurate superposition was achieved by aligning anatomic landmarks, such as the center of the optic disc and the fovea, with the corresponding perimetric landmarks, such as the center of the blind spot and fixation. In addition, three-dimensional surface plots were generated. These data were also used to calculate the mean and the maximum threshold elevation from baseline.

Images of FAF were recorded using a cSLO (Zeiss prototype SM 30-4024, Carl Zeiss Meditec, Jena, Germany), as has been described.<sup>13-16</sup> Briefly, FAF images were recorded with a  $40^\circ$  field-of-view mode and a confocal aperture that provided a full-width at half-maximum depth resolution of less than  $600 \mu\text{m}$ . The ametropic corrector was used to correct for refractive errors and to focus on the macula. An argon laser (488 nm) was used for illumination, and a wide band-pass filter with short-wavelength cutoff of 521 nm was inserted in front of the detector to record fundus FAF. A series of images of each eye were recorded at standard video scanning rates on SVHS videotape. Later, images were digitized at  $768 \times 576$  resolution using a frame grabber (Millennium; Matrox Imaging Products Group, Quebec, Canada). Thirty-two individual images were automatically aligned and averaged to reduce noise by using a published technique.<sup>30</sup> As a result, a single image was obtained for each study eye. A recent study has shown good within-session reproducibility and moderate interobserver reproducibility using the same system (Carl Zeiss Meditec) for FAF imaging.<sup>21</sup>

For the investigation of fixation, the cSLO was used in a way similar to that previously described.<sup>31</sup> It includes a helium-neon (He-Ne, 633 nm) laser that is modulated through an acousto-optic modulator as the primary source for producing the fixation stimulus. As the stimulus for fixation, a small cross with positive contrast was used. The system had been modified by the addition of an infrared diode (IR, 830 nm) laser. The IR laser allowed high-contrast images of the subject's fundus to be acquired. To examine fixation stability, the patient was asked to look

at the fixation cross. After approximately 10 seconds of fixation, we obtained images of the patient's fundus throughout a period of fixation of approximately 20 seconds. The images were stored on videotape (SVHS). The images were then digitized in sequence of 250 images at a rate of 25 per second. The digitized images were reviewed, and images of poor quality (e.g., affected by blinks) were discarded. As a result, approximately 10 seconds of fixation were sampled. One image of the sequence, usually the first, was taken as the master, to which the other images were aligned digitally by custom software. The alignment procedure provided the measurement of image mismatch on both the  $x$ - and  $y$ -axes. These  $x$ - and  $y$ -values reflect the eye movements and were used to calculate the bivariate contour ellipse area (BCEA).<sup>32,33</sup> This two-dimensional ellipse that describes the portion of retinal surface within the center of the target was imaged 68% of the time. The calculation of the BCEA enabled us to quantify the fixation stability of the patients and to compare the data with the existing literature.

Fine matrix mapping results were compared with age-matched data that have been published.<sup>23-25</sup> The individual threshold or luminance sensitivity data were also analyzed intraindividually in each patient, and sensitivity over areas of normal, increased, and decreased FAF was compared.

## RESULTS

FAF images were reviewed in 436 ARM patients from the Moorfields ARM database. Significant lens opacities may impair the quality of FAF images,<sup>21</sup> and thus a subset of the ARM patients whose lens opacities exceeded mild nuclear sclerosis on slit lamp examination were excluded. Moreover, a substantial number of the patients with ARM had foveal changes that precluded a visual acuity of at least 20/40. Altogether, 38 patients with ARM met the inclusion criteria. These 38 were contacted and invited to participate. Of those, seven were recruited for the study. Four female and three male patients were included (mean age, 75 years; range, 68-80 years). The predominant fundus features of the seven study eyes included extrafoveal CNV (two eyes), extrafoveal GA (two eyes), and soft drusen (three eyes). Median visual acuity in the seven study eyes was 20/25 (range, 20/40-20/20). The clinical data of the seven study eyes and the fellow eyes are presented in Table 1. The FAF images of the seven study eyes and one normal subject are shown in Figure 1.

Figure 2 shows the results of photopic and scotopic fine matrix mapping in a normal subject (not age-matched). Two separate sets of fine matrixes were tested: The first one was centered on the fovea (Figs. 2A-D), and the second one was placed on the nasal upper quadrant with the fovea on the lower temporal corner of the test grid (Figs. 2E-H). The left column shows photopic data, the right scotopic data. The first and third rows show luminance sensitivity contour plots that were superimposed on the FAF image shown in Figure 1H. The sensitivity profiles are shown with contour steps of 0.1 log unit. Corresponding three-dimensional surface plots of thresholds are shown below in the second and fourth rows (for the correspondence between the orientation of the edges of the contour plots and the three-dimensional surface plots, see legend to Fig. 2). In addition, Figure 2J shows a scotopic FMM centered on the fovea of an age-matched normal subject that represents the median of the control population when the mean threshold elevation from baseline is considered. The comparison between this data set compared with that shown in Figure 2D provides an estimate of the age effect for the scotopic threshold data (see also caption to Table 2). (For a similar comparison of the photopic data see Fig. 6E in Ref. 23 and the caption to Table 2.)

The data derived from both the scotopic and photopic FMM testing in all seven study eyes are summarized in Table 2. The

TABLE 1. Clinical Characteristics of the ARM Group

Patient	Age	Sex	Eye	FMM	Study Eye	Fellow Eye	VA (ETDRS)	VA Fellow Eye
1	68	M	L	Upper nasal macular quadrant	Extrafoveal CNV; s/p laser treatment	Few drusen	20/20	6/5
2	77	M	R	Lower nasal macular quadrant	Juxtafoveal CNV with disciform scar	Few RPE pigmentary changes; no drusen	20/40	6/6
3	69	F	R	Lower temporal macular quadrant	Secondary GA after longstanding PED; s/p prophylactic drusen laser; cellophane retinopathy	Large disciform macular scar after RPE tear	20/25	6/60
4	78	F	L	Central macular FFM	Extrafoveal GA; RPE hyperpigmentations; drusen	Subfoveal CNV with disciform scar and PED	20/25	6/24
5	80	M	L	Central macular FFM	Extensive drusen at the posterior pole including a large foveal druse	Subfoveal CNV with disciform scar	20/25	HM
6	79	F	R	Upper temporal macular quadrant	Large soft and small hard macular drusen	Subfoveal CNV with large disciform scar	20/20	HM
7	73	F	L	Lower temporal macular quadrant	Intermediate and large soft and small hard drusen	Subfoveal CNV with PED	20/20	6/36

The first four columns provide individual patient data. The fifth column indicates where the FMM grid was placed on the macula. The sixth and seventh column shows the predominant fundus features of the study eye and the fellow eye, respectively. The eighth column provide the visual acuity of the study eye determined with ETDRS charts. The last column shows the Snellen visual acuity of the fellow eye.

global estimates of the FMM, the mean (background), and the maximum (discrete) threshold elevation from baseline were derived from the Gaussian-filtered threshold data. Single sensitivity data for individual patients and their correspondence to FAF findings are described in later sections.

### Patients with CNV

In patient 1, extrafoveal CNV developed in the left eye 4 years before the study. He underwent laser treatment with an argon yellow laser (23 spots, 200  $\mu\text{m}$ , 0.5 sec, 260 mW), and the eye has been stable since then. FAF imaging (Fig. 1A) shows a lesion nasal to fixation, which exhibited decreased FAF, but was surrounded by increased FAF in the superior and inferior portion and both focally decreased and increased FAF in the nasal portion. Figure 3 shows the results of the photopic (left column) and scotopic (right column) FMM. The photopic FMM shows well-preserved foveal sensitivity. The lesion with decreased FAF showed a marked loss in sensitivity. However, the sensitivity loss was relatively uneven over the lesion, including only moderate sensitivity loss at some locations and nondetectable thresholds at other locations. The adjacent area on the nasal side of the lesion with focally decreased and increased FAF showed mild to moderate photopic sensitivity loss of approximately 1 log unit. The area of increased FAF adjacent to the lesion on the superior side showed near normal sensitivity, which was sharply demarcated toward the inferior lesion. Scotopic FMM revealed severely reduced sensitivity over the lesion with decreased FAF. However, the scotopic sensitivity was also extremely reduced over both the area of increased FAF (superior portion) and over the area with both focally decreased and increased FAF (nasal portion). The scotopic sensitivity over areas with normal FAF that were adjacent to the areas of abnormal FAF was only mildly reduced.

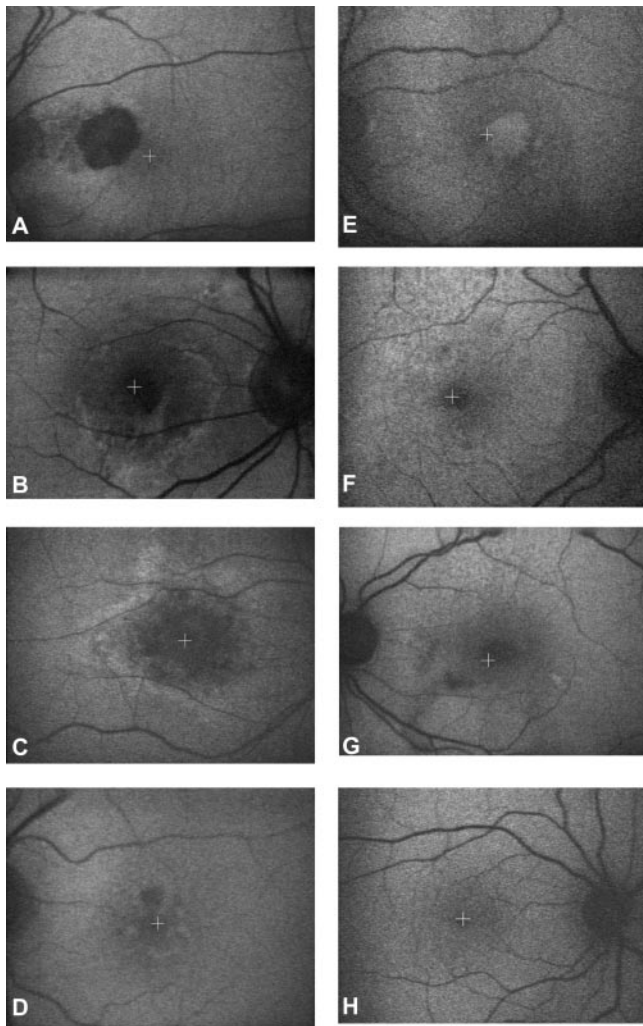
In patient 2, extrafoveal CNV developed in the right eye 15 months before the study. Intravenous fluorescein angiography revealed occult CNV. Visual acuity has been stable since then without intervention. The fundus showed a disciform scar with foveal sparing surrounded by a halo of RPE atrophy. FAF imaging showed centrally decreased FAF (Fig. 1B). The central area of mainly decreased FAF was surrounded by a ring of increased FAF at approximately 1000  $\mu\text{m}$  eccentricity. Approx-

imately 750  $\mu\text{m}$  inferior to fixation, there was an additional small area of increased FAF. Photopic FMM (Fig. 4) revealed severely reduced sensitivity in the foveal region and moderately reduced sensitivity in the central area, with mainly decreased FAF. The ring of increased FAF was not different in sensitivity from the more eccentric portion of the test field and showed only mildly reduced sensitivity. Scotopic FMM revealed complete sensitivity loss, including both the central area of decreased FAF and the surrounding ring of increased FAF. More eccentrically, the sensitivity was still considerably reduced (by approximately 2 log units) but was also significantly better preserved than in the area of increased FAF.

### Patients with GA

Patient 3 underwent prophylactic laser treatment for confluent soft drusen in the right eye 5 years before the study (12 spots, 200  $\mu\text{m}$ , 0.2 s, 80 mW). She then experienced a longstanding RPE detachment (PED). Subsequently, secondary GA and some degree of cellophane retinopathy developed. FAF imaging showed a central area of decreased FAF with a radius of approximately 1000  $\mu\text{m}$  surrounded by a ring of increased FAF (Fig. 1C). Photopic FMM revealed normal foveal sensitivity and mildly to moderately reduced sensitivity over the adjacent area of decreased FAF, but normal sensitivity over the area of increased FAF and also normal photopic sensitivity eccentric to the area of increased FAF (Fig. 5). Scotopic FMM showed a complete loss of sensitivity over both the central area of decreased FAF and the surrounding ring of increased FAF. Eccentric to the area of increased FAF, the scotopic sensitivity was moderately reduced by approximately 1 log unit.

Patient 4 exhibited a small patch of GA approximately 500  $\mu\text{m}$  superior to fixation with a diameter of approximately 300  $\mu\text{m}$  in the left eye. FAF imaging showed an area of decreased FAF, surrounded by a ring of increased FAF, confirming GA of the RPE (Fig. 1D). Temporal, nasal, and inferior to fixation, there were additional patches of increased FAF at approximately 500  $\mu\text{m}$  eccentricity. Photopic FMM did not reveal reduced sensitivity (Table 2; single sensitivity data not shown). Scotopic FMM, however, showed extremely reduced sensitivity including the fovea, the area of decreased FAF (GA), and the areas of increased FAF. More eccentric to the areas of increased



**FIGURE 1.** FAF images of the seven study eyes. The central *crosshair* indicates the fixation location of the individual patient, which was revealed by the investigation of fixation stability. Images from patients (A) 1, (B) 2, (C) 3, (D) 4, (E) 5, (F) 6, and (G) 7 are shown. For comparison, a normal FAF image (H) is shown derived from the normal subject whose data are presented in Figure 2.

FAF, the scotopic sensitivity was better preserved but still moderately reduced.

### Patients with Drusen

Patient 5 exhibited extensive drusen at the posterior pole, including a large, soft foveal druse. FAF imaging revealed a central area of increased FAF corresponding to the large, soft foveal druse (Fig. 1E). There were some additional small patches of increased FAF at the posterior pole that corresponded to the drusen distribution. Photopic FMM revealed a mildly reduced sensitivity over the test field of approximately 0.5 log units (Fig. 6). The sensitivity decrease over the area of increased FAF was slightly larger. Scotopic FMM revealed considerably reduced sensitivity over the area of increased FAF. This sensitivity decrease extended the area of increased FAF by approximately  $1^\circ$  to  $2^\circ$  inferiorly and temporally. The remainder of the test field showed moderately reduced scotopic sensitivity.

Patient 6 showed many large, soft and small, hard drusen at the posterior pole of the right eye. FAF imaging showed an area of reticular increased FAF in the superior portion of the macula

that poorly corresponded to drusen distribution (Fig. 1F). Photopic FMM revealed mildly reduced sensitivity of approximately 0.5 log unit that was equally distributed over the test field (Table 2; single sensitivity data not shown). Scotopic FMM showed moderately reduced sensitivity over the areas with reticular increased FAF. At the few test spots outside the area of abnormal FAF, the scotopic sensitivity was near normal.

In patient 7, only data for photopic sensitivity testing were available. The patient exhibited intermediate and large, soft drusen and small, hard drusen at the posterior pole. FAF imaging showed a small patch of increased FAF in the inferior temporal macula that corresponded to a large druse (Fig. 1G). Otherwise, FAF and drusen distribution did not correspond. Photopic FMM did not reveal any significant sensitivity loss over the inferior temporal test field (Table 2; single sensitivity data not shown).

### Fixation Stability

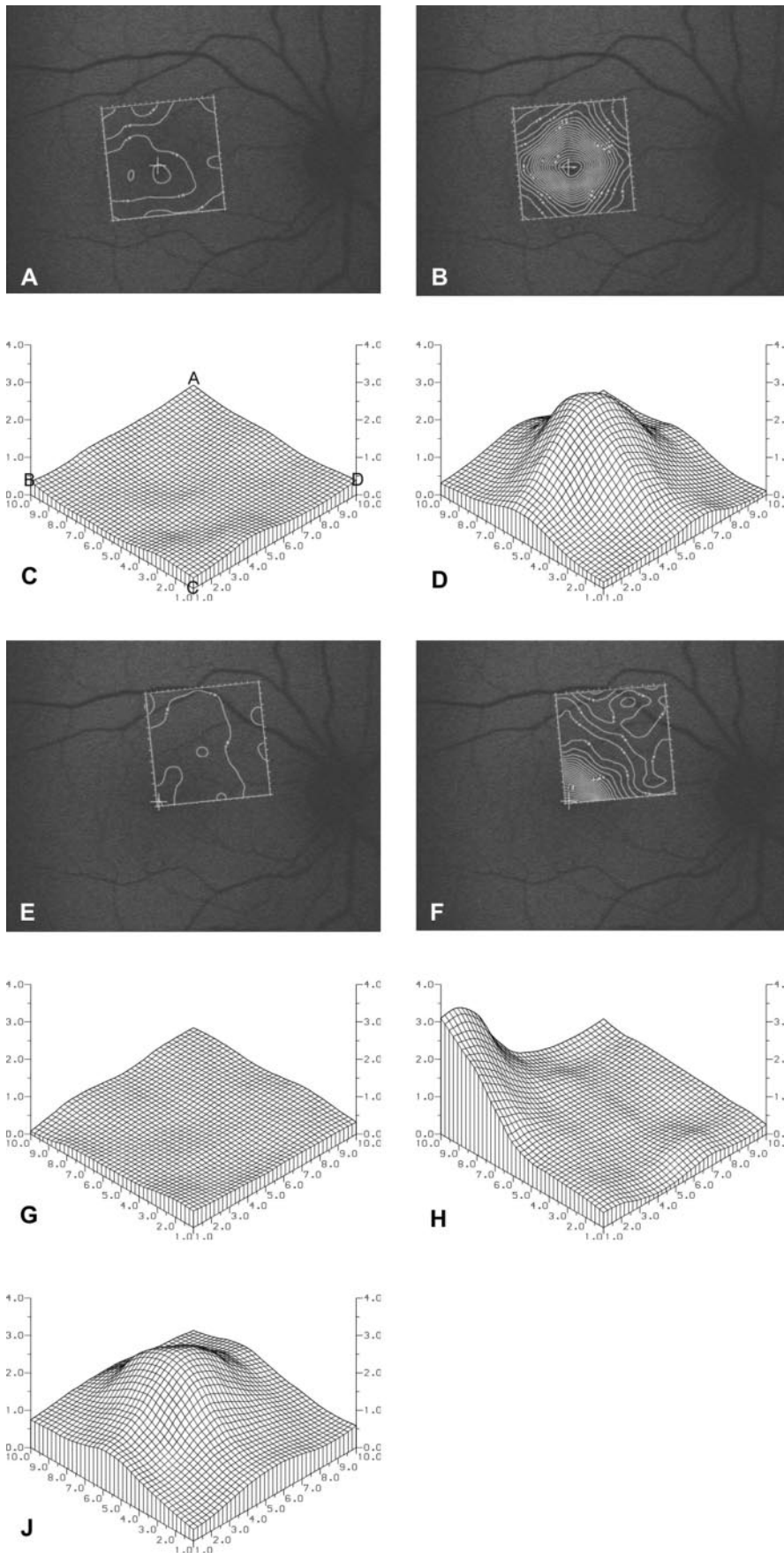
The fixation locations of all patients with ARM studied are shown in Figure 1. Quantitative fixation stability testing by determining the BCEA was not performed in patient 2. The BCEAs are presented in Table 2. All the patients (including patient 2) used a single retinal locus for fixation, which was confirmed by observing the patient's fundus throughout testing. There was considerable interindividual variability of the BCEA. The mean BCEA was 501 minarc<sup>2</sup> (range, 253–862 minarc<sup>2</sup>). This means that fixation was quantitatively very stable in four of six patients (below 550 minarc<sup>2</sup>), and was still reasonably stable in patients 1 and 7 (862 minarc<sup>2</sup> and 742 minarc<sup>2</sup>, respectively). Qualitative assessment of the fixation stability of patient 2 also revealed very stable fixation.

### DISCUSSION

The areas of increased FAF in this group of patients with ARM indicated moderate to severe scotopic sensitivity loss. Photopic sensitivity was consistently less reduced. It was either normal or showed a minor decrease. Thus, in all six patients with ARM in whom scotopic and photopic FMM was available, rod sensitivity loss exceeded cone sensitivity loss. We suggest that increased FAF in ARM has a functional correlate and it indicates preferential dysfunction of the rod system.

There was a considerable interindividual variability in fixation stability. However, when compared with BCEAs in normal subjects in previous studies in which a cSLO was used, the fixation stability of all patients was within normal limits.<sup>31,34</sup> This is in line with expectations, in that there is no strong correlation between fixation stability and age and because patients with macular lesions who still use the fovea for fixation may have relatively normal fixation.<sup>31</sup> We cannot exclude, however, that the relatively lower fixation stability in patients 1 and 7 impeded the detection of photopic or scotopic dysfunction within the area of increased FAF. We conclude that abnormal macular FAF in ARM does not imply fixation instability. The fixation data underline the reliability of our findings that correlate luminance sensitivity with fundus abnormalities.

The evidence that FAF is derived largely from lipofuscin within the RPE comes from observations by Delori et al.,<sup>10,11</sup> who showed that the signal was derived from between the neurosensory retina and the choroid and from the spectral characteristics of lipofuscin. Moreover, variation of the levels of FAF was compatible with histopathologic findings in aging and disease.<sup>11</sup> However, the functional influence of focal lipofuscin accumulation within the RPE to retinal disease is still a matter of debate. Holz et al.<sup>35</sup> prospectively studied areas of increased FAF associated with GA and found that within these areas of increased FAF, new atrophic areas developed. They



**FIGURE 2.** Photopic (A, C, E, G; *left column*) and scotopic (B, D, F, H; *right column*) data derived from FMM that was centered on the fovea; (A–D) data derived from FMM that was centered on the fovea; (E–H) FMM of the nasal upper quadrant with the fovea on the lower temporal corner of the test grid. For correspondence between the orientation of the luminance sensitivity contour plots on the FMM images and the three-dimensional threshold surface plots, letters on the FMM in (C) are added. In all FAF contour maps shown, A corresponds to the *bottom right* corner, B to the *bottom left* corner, C to the *top left* corner, and D to the *top right* corner, respectively. (J) For comparison to the FMM in (D), a scotopic FMM centered on the fovea is shown from an age-matched normal subject that represents the median of this control population when the mean threshold elevation from baseline is taken into account (derived from previously published data<sup>24,25</sup>). The comparison between (D) and (J) gives an estimate of the age effect on the scotopic threshold data (see also Fig. 3A in Ref. 24). For the comparison between the photopic three-dimensional threshold surface plot (C) and an age-matched population see Figure 6E in Ref 23.

**TABLE 2.** The Global Estimates of the FMM

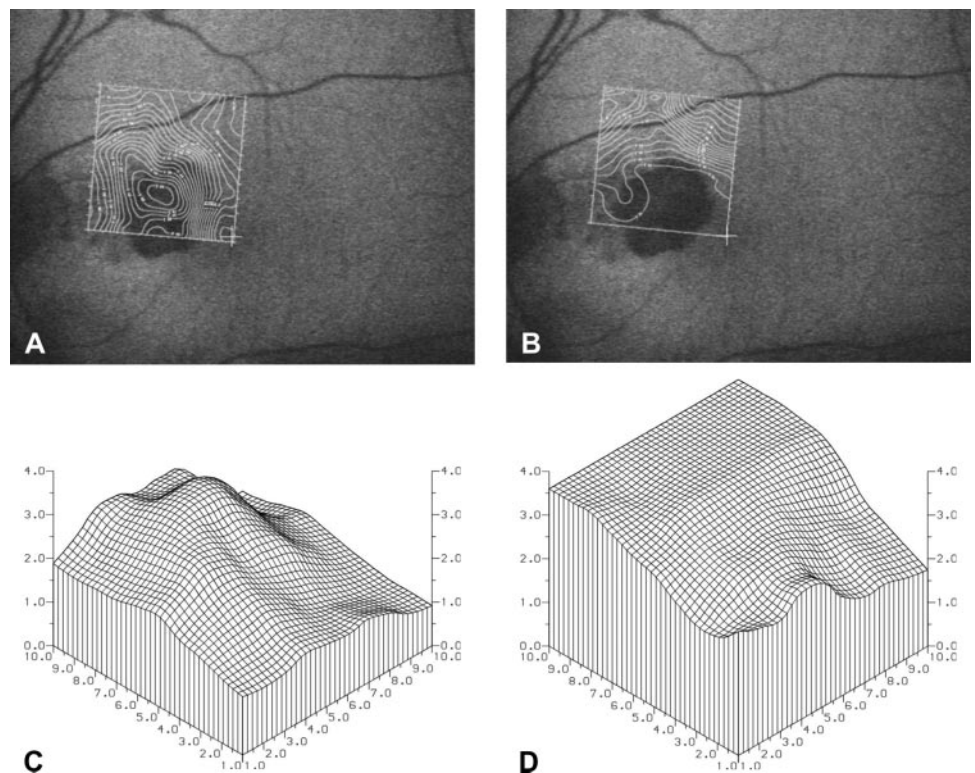
Patient	Photopic FFM		Scotopic FFM		Fixation Stability (BCEA)
	Background Threshold (log)	Discrete Elevation (log)	Background Threshold (log)	Discrete Elevation (log)	
1	1.87	3.05	3.13	3.60	862
2	1.92	3.11	3.46	3.63	NP
3	1.08	2.31	2.84	3.60	279
4	0.97	1.28	2.53	3.58	549
5	1.44	2.06	2.62	3.58	253
6	1.39	1.64	1.99	3.59	320
7	0.46	0.65	NP	NP	742

Shown are the mean (background) and the maximum (discrete) threshold elevation from baseline in all seven study eyes (log units). These global estimates were derived from the Gaussian-filtered threshold data. For comparison, the mean and maximum photopic threshold elevation derived from the FMM centered on the fovea in the normal subject (Fig. 2C) was 0.27 log unit and 0.51 log unit, respectively. The mean and maximum photopic threshold elevation derived from the FMM placed on the nasal upper macular (Fig. 2G) was 0.31 log unit and 0.47 log unit, respectively. The mean and maximum scotopic threshold elevation derived from the FMM centered on the fovea in the normal subject (Fig. 2D) was 0.76 log unit and 2.46 log units, respectively. The mean and maximum scotopic threshold elevation derived from the FMM placed on the nasal upper macular (Fig. 2H) was 0.69 log unit and 3.11 log units, respectively. The mean and maximum scotopic threshold elevation derived from the FMM in the age-matched normal subject (Fig. 2J) was 1.04 log units and 2.36 log units, respectively. The last column provides the BCEA values (min arc<sup>2</sup>) derived from fixation stability testing. For comparison, the BCEA value for the normal subject (Fig. 2A–H) was 224 min arc<sup>2</sup>.

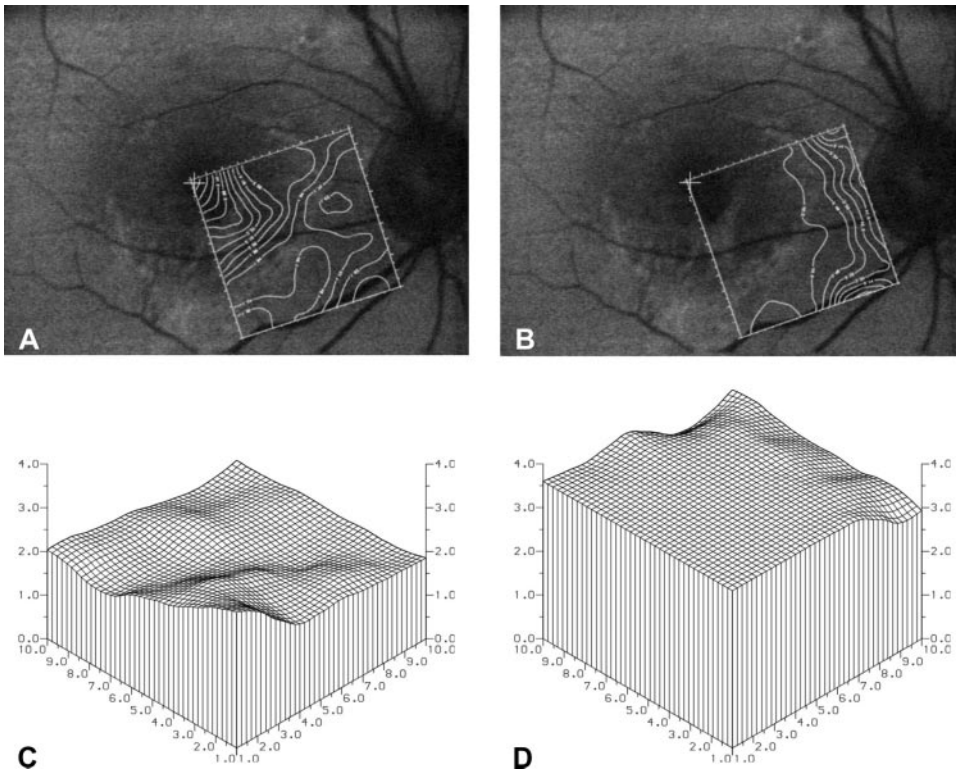
suggested that areas of increased FAF precede the development and enlargement of GA in ARM. The observation that the number of photoreceptor cells is reduced in the presence of increased lipofuscin content in the RPE led to the proposal that the increased accumulation of autofluorescent material may occur before cell loss.<sup>36</sup> Our finding of considerable scotopic sensitivity loss over areas of increased FAF further elucidates the significance of FAF in ARM. In view of the different underlying diseases in our patients (including CNV, GA, and drusen) the consistency of scotopic sensitivity loss exceeding photopic sensitivity loss was remarkable. However, it is not clear whether the primary event occurs in the RPE and subsequently affects photoreceptor function or if the inductive event is in the photoreceptors themselves and the RPE is subsequently

affected. Longitudinal studies of both FAF and rod and cone photoreceptor function are needed to resolve this question.

In previous studies, a lack of obvious correspondence between the distribution of drusen and FAF has been found.<sup>14,16</sup> Although it is widely believed that the material deposited into Bruch’s membrane is derived from the RPE, the correlation between the two is not close.<sup>17</sup> In this study, we also observed that the distribution of drusen and FAF correlated poorly and that photoreceptor dysfunction deficits followed the distribution of both increased and decreased FAF but did not follow the distribution of drusen. In previous psychophysical studies including both scotopic and photopic sensitivity testing, no correlation between drusen and photoreceptor dysfunction was found,<sup>37–39</sup> suggesting that drusen distribution on the



**FIGURE 3.** Photopic and scotopic FMM in patient 1 (extrafoveal CNV; status post laser treatment). *Left column:* photopic FMM; *right column:* scotopic FMM; *top row:* photopic (A) and scotopic FMM (B) contour plots superimposed to the FAF image (shown in Fig. 1A) that indicate the size and location of luminance sensitivity gradients; *bottom row:* three-dimensional surface plots that indicate Gaussian-filtered threshold data for photopic (C) and scotopic (D) sensitivity testing.

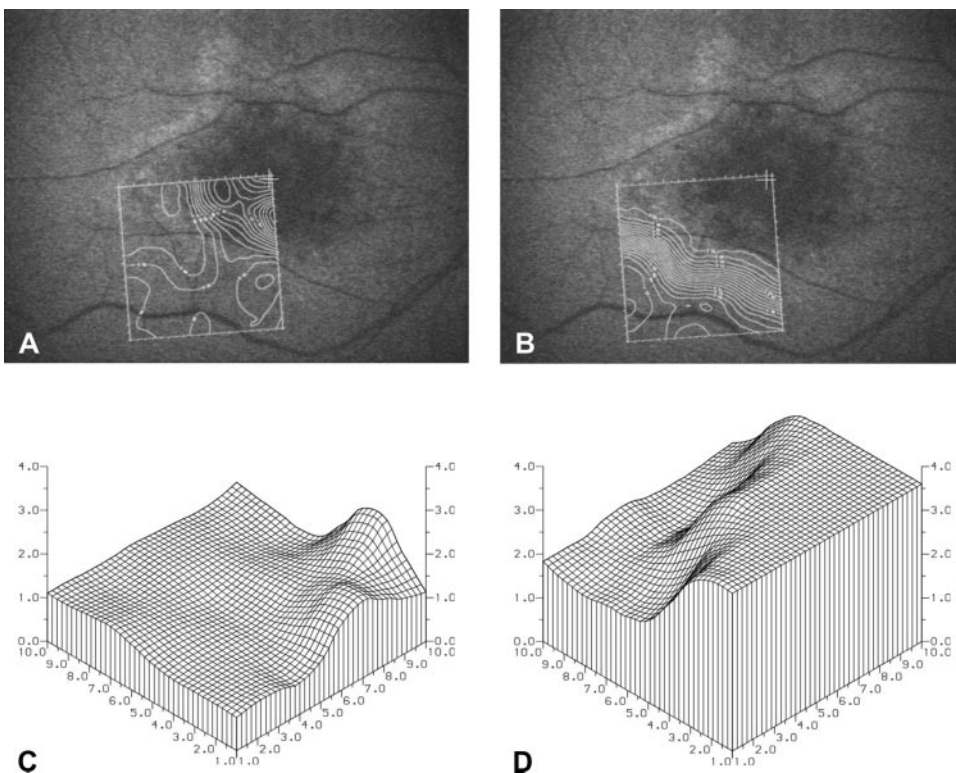


**FIGURE 4.** Photopic and scotopic FMM in patient 2 (extrafoveal CNV with disciform scar). The panels are assembled as in Figure 3.

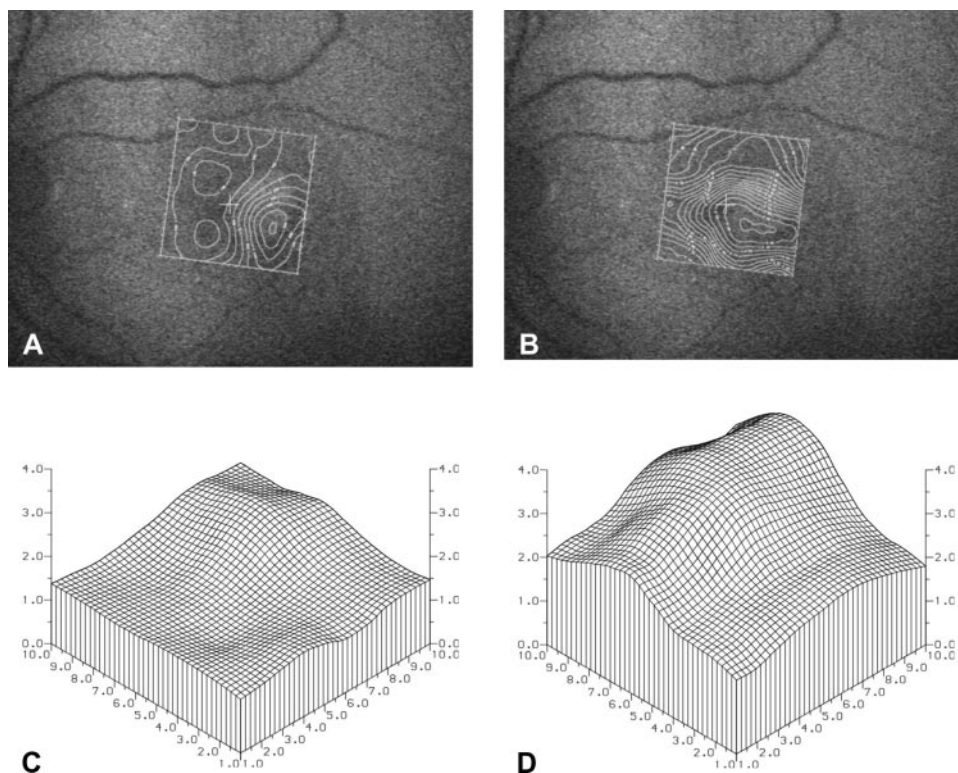
fundus and photoreceptor function tests measure different expressions of the ARM process. We conclude that drusen and FAF have different functional implications, and we support the hypothesis that they represent two independent measures of aging and ARM in the retina.<sup>16</sup>

The exception to this finding was the large, soft foveal druse (patient 5). We confirm that large, soft foveal drusen

appear to correspond to areas of increased FAF, as has been observed recently.<sup>16</sup> Moreover, the area covered by the large, soft druse showed mildly reduced photopic and considerably reduced scotopic sensitivity. Given that large, soft foveal drusen represent small RPE detachments, it is not surprising that scotopic sensitivity was affected. Similarly reduced scotopic sensitivity has been found in patients with central



**FIGURE 5.** Photopic and scotopic FMM in patient 3 (secondary GA after longstanding RPE detachment; s/p prophylactic laser treatment). The panels are assembled as in Figure 3.



**FIGURE 6.** Photopic and scotopic FMM in patient 5 (large, soft foveal druse). The panels are assembled as in Figure 3.

serous retinopathy.<sup>26</sup> Our observation reinforces the impression that large, soft foveal drusen are somewhat different from large, soft drusen elsewhere.<sup>16</sup>

Our data are in accord with recent functional studies that showed that the rod system is preferentially affected in aging and ARM. Older patients with a normal fundus appearance have scotopic impairment greater than photopic impairment in 80% of cases, and, furthermore, scotopic sensitivity throughout adulthood declines faster than does photopic sensitivity.<sup>40</sup> In a recent psychophysical study it was found that in early ARM there can be prominent dark-adapted dysfunction.<sup>37</sup> The mean scotopic sensitivity at the posterior pole was significantly lower in early ARM patients as a group than in age-matched normal subjects. The topography of sensitivity loss at the posterior pole varied considerably among individual patients with early ARM. In almost all (87%) the patients who exhibited reduced light sensitivity, the magnitude of mean scotopic sensitivity loss exceeded the magnitude of mean photopic sensitivity loss.<sup>37</sup>

The preferential vulnerability of the rod system in aging and ARM has also been shown by histologic studies. In maculae of older adults without grossly visible drusen and pigmentary change (i.e., they do not have ARM), the number of cones in the cone-dominated part of the macula remained stable at approximately 32,000 through the ninth decade.<sup>41</sup> In contrast, the number of rods in the maculae of the same eyes decreased by 30%. The greatest loss occurred in the parafovea. With respect to photoreceptor topography at different stages of ARM, the foveal cone mosaic of eyes with large drusen and thick basal deposits appeared surprisingly similar to that of age-matched control eyes, and the total number of cones was normal.<sup>42</sup> In contrast, in the parafovea, cones appeared large and misshapen, and few rods remained. Furthermore, in eyes with late ARM, virtually all surviving photoreceptors in the macula were cones, a reversal of the normal predominance of rods. Preferential loss of rods over cones was found in three of four early and late ARM eyes examined.<sup>18</sup> We conclude that our finding of preferential rod sensitivity loss over areas of

increased FAF is in line with these histologic data although they do not readily explain the magnitude of sensitivity losses.

This dilemma has been addressed by others. Very recently, Curcio et al.<sup>18–20</sup> introduced the retinoid-deficiency hypothesis for the pathogenesis in ARM. It is widely thought that debris that accumulates with age in Bruch's membrane is derived from the RPE, which discharges cytoplasm contents into the inner portion of Bruch's membrane to achieve cytoplasmic renewal.<sup>43–45</sup> This material is thought to be cleared by the choroid, and that incomplete clearance causes thickening of Bruch's membrane. Thus, a relationship between accumulation of debris in the RPE, as shown by increased FAF, and in Bruch's membrane might be expected.<sup>14</sup> In patients with ARM with manifest choroidal perfusion abnormality, scotopic FMM showed discrete areas of scotopic sensitivity loss that corresponded closely to regions with prolonged choroidal filling, whereas age-matched control eyes did not have scotopic sensitivity loss. It was hypothesized that diffuse deposits of abnormal material might account for both the perfusion abnormality and functional loss by acting as a diffusion barrier between the choriocapillaris and the RPE.<sup>22</sup> The visual cycle comprises biochemical reactions in the RPE and photoreceptors that produce the vitamin A derivative 11-*cis*-retinal from all-*trans* precursors derived from the extracellular space of the outer retina and delivered across Bruch's membrane by plasma proteins.<sup>46,47</sup> Not only is 11-*cis*-retinal necessary to regenerate the photoreceptor pigment after bleaching by light, but retinoids are also required for photoreceptor survival. Vitamin A deprivation leads to outer segment degeneration and photoreceptor death *in vivo*.<sup>48–50</sup> The vulnerability of vitamin A availability with thickening of Bruch's membrane is illustrated by the reversal of sensitivity loss in Sorsby fundus dystrophy by the supplementation by vitamin A alone.<sup>51</sup> In ARM, lack of vitamin A may be due to change in the diffusion characteristic of Bruch's membrane,<sup>52</sup> or to the inability to recycle products of phagosomal degradation in the RPE,<sup>53</sup> or to a combination of the two. Lack of vitamin A affects primarily rods but eventually affects cones as well.<sup>54–56</sup> Relative retinoid deficiency caused



by ARM-related disease at the level of the RPE and Bruch's membrane would explain our observation that there is topographic correspondence of ARM-related photoreceptor dysfunction and RPE dysfunction reflected by increased FAF. Moreover, there is suggestive evidence that rod and cone photopigments regenerate by different mechanisms. In frog retinas separated from the RPE, cone opsin, but not rhodopsin, regenerates spontaneously.<sup>57,58</sup> Mata et al.<sup>59</sup> very recently found several catalytic steps of an alternate visual cycle that mediates pigment regeneration in cones. This alternate pigment regeneration is independent from the RPE and may involve Müller cells, which implies that visual pigment regeneration in cones is not exclusively dependent on RPE function in contrast to the rod system. We hypothesize that relative retinoid deficiency due to RPE dysfunction as reflected by abnormal FAF explains the higher susceptibility of the rod system relative to the cone system that we observed in our study.

In this cross-sectional study of photopic and scotopic sensitivity in ARM heterogeneous phenotypes of this disease were included. Moreover, one patient with CNV received laser treatment, and thus this case does not necessarily represent the natural history of ARM. We conclude that investigations should be expanded and further studies should be performed, especially longitudinal studies, to confirm our findings and to see whether the questions raised and the hypotheses generated herein can be solved and verified, respectively. Such studies should also include subjects who are either normal or have only a few drusen and who are matched for age and gender.

Our findings may have implications for future intervention to maximize cone survival in ARM. Rod photoreceptors serve as an early indicator of impending cone dysfunction and they contribute to preserve the later-degenerating cones, because rods produce a diffusible substance essential for cone survival.<sup>60-62</sup> Our study shows that areas of abnormal FAF can have considerably reduced scotopic sensitivity and that this does not necessarily represent an early sign of ARM. However, because cone photoreceptor function was either normal or only mildly reduced, this relatively simple imaging technique may serve to indicate an appropriate disease stage for early intervention to save the cone system.

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