Fundus Autofluorescence and Fundus Perimetry in the Junctional Zone of Geographic Atrophy in Patients with Age-Related Macular Degeneration

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Purpose. To investigate retinal sensitivity in the junctional zone of geographic atrophy (GA), with variations in fundus autofluorescence (FAF) in patients with advanced age-related macular degeneration (AMD).

Methods. The spatial distribution and intensity of FAF were recorded with a confocal scanning laser ophthalmoscope (SLO). Eyes had normal background FAF (group 1) or increased FAF (group 2) surrounding the atrophic patches. Retinal sensitivity was assessed by applying light stimuli with static automated full-threshold fundus perimetry with a modified SLO. Threshold sensitivities were compared with age-matched normal sensitivities.

Results. Thirty-nine eyes of 39 patients with GA were included. Group 2 had a higher percentage of all test points outside the GA area, with decreased retinal sensitivity (44.9% ± 28.7%) compared with group 1 (20.7% ± 12.7%; $P = 0.0063$; multiple regression model; outcome variable is retinal sensitivity; covariates are group affiliation and GA area). Within group 2, the average percentage of stimuli in areas of normal FAF with reduced sensitivity was 38.0% ± 33.0%, whereas the average percentage of stimuli in areas of elevated FAF with reduced sensitivity was 52.6% ± 29.7% ($P = 0.023$, Wilcoxon signed rank test).

Conclusions. Areas of increased FAF outside GA may be associated with variable degrees of loss of retinal sensitivity and suggest a functional correlate of excessive accumulation of retinal pigment epithelium lipofuscin in AMD. Combining in vivo recording of FAF and retinal sensitivity, using SLO technology, may give important clues in the understanding of mechanisms of disease. (*Invest Ophthalmol Vis Sci.* 2004;45:4470–4476) DOI:10.1167/iovs.03-1311

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Age-related macular degeneration (AMD) is the most common cause of irreversible loss of central vision and legal blindness in developed countries.[1–3] Besides choroidal neovascularization, geographic atrophy (GA) of the retinal pigment epithelium (RPE) is a frequent cause of severe visual loss in patients with AMD.[4–7] The pathophysiologic mechanisms underlying the atrophic process, which involves not only the RPE but also the outer neurosensory retina and the choriocapillaris, are poorly understood.

In human postmitotic RPE cells, lipofuscin (LF) accumulates with age within the lysosomal compartment. It is mainly derived from the chemically modified residues of incompletely digested photoreceptor outer segment discs.[11,12] Recent experimental findings suggest that certain molecular compounds of LF, such as A2-E possess toxic properties and may interfere with normal cell function.[13–15]

LF accumulation has been studied largely in vitro with fluorescence microscopic techniques. Several studies have shown that fundus autofluorescence (FAF) in vivo is mainly derived from LF in the RPE.[8,9,15–17] With the advent of scanning laser ophthalmoscopy (SLO) it is possible to document FAF and its spatial distribution and intensity over large retinal areas in vivo, as initially described by von Rückmann et al.[18,19] and others.[20,21]

With the application of this technique, various patterns of increased FAF in the junctional zone of GA have been identified in patients with AMD (Schmitz-Valckenberg S, et al. *IOVS* 2002;43:ARVO E-Abstract 2518).[22,23] Furthermore, it has been shown that such areas of increased FAF may precede the enlargement of preexisting atrophy and the development of new atrophic patches over time.[23]

Fundus perimetry (FP) using SLO technology allows for exact correlation between fundus changes and functional impairment.[24–27] Because areas of GA are readily identified by the infrared light source of the SLO, it is possible to determine and compare retinal threshold sensitivities in and around areas of GA. To investigate the functional implications of FAF changes surrounding atrophic patches in eyes with advanced atrophic AMD, we tested retinal sensitivity in these areas with FP.

Patients and Methods

Patients with GA secondary to AMD were consecutively recruited in the Department of Ophthalmology at the University of Heidelberg as part of a natural history study (the Fundus Autofluorescence in Age-related Macular Degeneration [FAM]) Study). Each patient underwent a routine ophthalmic examination, including funduscopy and determination of best corrected central visual acuity with Early Treatment Diabetic Retinopathy Study (ETDRS) charts. Pupils were dilated with 1% tropicamide before FAF and FP examinations. Only patients >50 years of age, with clear media that allowed FAF imaging and with uni- or multifocal GA of the RPE were included. If GA was present in both eyes, one eye per patient was randomly chosen for evaluation. Exclusion criteria included any history of retinal surgery, laser photocoagulation, or radiation therapy or other retinal diseases in the study eye,

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including diabetic retinopathy and hereditary retinal dystrophies. Fluorescence or indocyanine green angiography was performed only if there were funduscopic signs present that were indicative of neovascular AMD in addition to patches of GA. Such eyes were excluded from the study. FAF was measured with a confocal SLO (Heidelberg Retina Angiograph [HRA]; Heidelberg Engineering, Dossenheim, Germany), the optical and technical principles of which have been described previously.[28] Briefly, an argon blue laser (488 nm) is used for excitation, and the emitted light above 500 nm is detected with a barrier filter. The reflectance of the blue argon laser light is suppressed by a factor of 10^{-6} with an interference filter. Consequently, it is assumed that the reflectance signal does not contribute to the obtained FAF image. The rectangular field of view was adjusted to 30° × 30°. A standardized protocol for FAF image acquisition using the HRA included sensitivity adjustment so that retinal vessels and optic disc were optimally visible, focus of the retinal image in reflection mode before FAF imaging, imaging of the entire macular area and the optic disc, acquisition of at least 15 single 30° FAF 512 × 512-pixel images in series mode, and selection of the best nine images for automatic alignment and calculation of mean images. The total size of atrophy was measured by manual outlining with image analysis software (Heidelberg Eye Explorer; Heidelberg Engineering). If there was more than one area of atrophy in an eye, total size represented the sum of all atrophic areas.

Atrophic areas typically show a markedly reduced FAF signal due to the absence of RPE and thus indicate the absence of autofluorescent LF.[19,21] As previously described, FAF may be normal or, in most eyes, show various patterns of increased autofluorescence in the junctional zone of GA.[22]

Automated static threshold FP was performed for each patient, with another SLO (model 101; Rodenstock, Ottobrunn, Germany) and customized software developed by our group, as described in detail earlier.[24–26] Background illumination was set to 10 cd/m^2. Stimuli comparable to Goldmann III size were presented for 120 ms with an automated 4-2-1 strategy for measuring light increment threshold. The interindividual variability with regard to extension and shape of the atrophic areas does not allow application of the same testing grid in all eyes examined. Therefore, the stimulus distribution was optimized for each case by the option to define individual stimulus locations according to the FAF image. Stimuli were positioned on atrophic areas and their junctional zone, including areas of increased FAF, if present. The fixation target was shifted, so that the atrophic area was centered in the examined area, when applicable. The results obtained from the examinations were compared with the age-related normal values from our database.[29] We evaluated the total number of stimuli as well as those positioned in areas of elevated FAF, as described later. In previous studies, particularly patients with open-angle glaucoma have shown that a single reduction of light increment sensitivity in one point of >4 dB allows for detection of scotomata.[30] To compensate for intra- and interindividual variances in perimetric testing, only values with a >4 dB threshold reduction in comparison to age-matched normal values were classified as pathologic. All areas with these values are regarded as representing decreased retinal sensitivity. Due to the nature of the subjective testing method, only patients with good parameters of cooperation (≤15% false-positive or false-negative answers) were included in the study. All examinations with the FP system were performed by the same investigator.

Statistical evaluation of the data for retinal sensitivities had to account for the individual fundus diseases. To test the relationship between different degrees of increased FAF and impaired visual function, we calculated the amount and the percentage of test points outside the atrophy with significant functional alterations compared with age-related normal values for each eye. Patients were divided into two groups based on the FAF findings. Group 1 encompassed eyes with normal background FAF outside the atrophy, whereas group 2 included eyes with abnormal increased FAF in the junctional zone. In the latter, stimuli within areas of elevated FAF and within areas of normal FAF were compared with test for differences in retinal sensitivity.

The data obtained were analyzed with frequency and descriptive statistics. Statistical analysis of age, GA area, and visual acuity of both groups were determined with the Wilcoxon rank sum test. For the comparison of retinal sensitivity, a multiple-regression model was fitted with retinal sensitivity as the outcome variable and group affiliation and GA area as the covariates. The analysis of retinal sensitivity within group 2 was performed with the Wilcoxon signed rank test. The statistical calculations were performed on computer (Pentium II computer with Windows 95 and SAS software, ver. 8.2; SAS Institute Inc., Cary, NC).

Maximum retinal irradiance of the lasers used for FAF imaging and for FP was well below the limits established by the American National Standards Institute and other international standards (ANSI Z136.1; 1993). The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Heidelberg. Before inclusion, written, informed consent was obtained from each participating patient after explanation of the nature of the study.

Results

Thirty-nine eyes of 39 patients (mean age, 74.5 ± 7.8 years) were consecutively recruited into the study when they met the inclusion criteria. Eleven patients had to be excluded from the study because of a high number of false-positive or false-negative test results during perimetry or because of very unstable fixation that made valid FP testing impossible. There were 14 eyes in group 1 and 25 eyes in group 2. Mean age was not significantly different between the two groups ($P = 0.463$).

In both groups, there was a wide range of visual impairment and total size of atrophy between individual eyes (Tables 1, 2). Overall visual acuity was not significantly different between the two groups ($P = 0.669$). In group 1, mean visual acuity was 10/40 ± 4.1 lines, whereas it was 10/50 ± 3.6 lines in group 2. In three eyes (two in group 1, one in group 2), the size of total atrophy could not be measured, because the atrophy exceeded the FAF image (30° × 30°). In group 1, the mean total area of atrophy was 4.45 ± 2.87 mm^2 and in group 2, 11.40 ± 7.66 mm^2 ($P = 0.002$).

The average total number of stimulus locations in FP per eye was 61.4 ± 16.0 in group 1 and 65.5 ± 20.4 in group 2. Examples of group 1 and group 2 are illustrated in Figures 1A–D with FP results, FAF image, and fundus photograph. The average number of stimuli with decreased retinal sensitivity in comparison to age-matched normal values was 11.1 ± 7.2 in group 1 and 25.4 ± 20.8 in group 2. The latter presented with a significantly higher percentage (Wilcoxon signed rank test, $P = 0.0074$) of all test points with reduced retinal sensitivity outside the atrophic area (44.9% ± 28.7%) compared with group 1 (20.7% ± 12.7%; Fig. 2A). Using a multiple-regression model, which considers the different sizes of the GA area in the groups, the difference between the two groups was statistically significant ($P = 0.0063$).

In an additional analysis, only the eyes of group 2 were examined. Light stimuli in areas with normal FAF were directly compared with light stimuli in areas with various degrees of increased FAF within eyes. As illustrated in the examples, no clear pattern of changes in retinal sensitivity between areas of normal FAF compared with areas with increased FAF at the junctional zone was observed. Different degrees of impaired retinal sensitivity were observed in retinal loci that showed similar FAF signals, whereas a similar decrease in retinal sensitivity was noted in retinal loci that had markedly different FAF signals.

A standardized testing grid during FP, as used for other applications, could not be used in the current one, because the

TABLE 1. Summary of Demographics and Results in Eyes with Normal FAF in the Junctional Zone of Atrophy in Group 1

No.	Eye	Age	VA	GA Area	*n*	*n* > 4 dB Loss	Percentage
1	R	62	10/40	6.06	59	11	18.6
2	R	71	10/16	4.53	57	9	15.8
3	L	63	10/32	6.70	60	7	11.7
4	R	79	5/100	0.96	46	16	34.8
5	R	66	10/20	NA	58	9	15.5
6	L	70	10/50	NA	32	14	43.8
7	R	74	5/125	2.32	40	5	12.5
8	L	78	10/80	2.32	32	7	21.9
9	R	83	10/32	1.44	73	6	8.2
10	L	74	10/100	9.49	52	4	7.7
11	L	73	5/100	5.35	66	1	1.5
12	L	78	10/16	2.25	77	22	28.6
13	L	66	10/25	8.90	68	26	38.2
14	R	91	10/32	3.10	58	18	31.0

Studied eye (No.), side (eye: R, right; L, left), age (y), visual acuity (VA), size of total atrophy (GA area, in mm^2), total number of test points outside GA (*n*), and number ($n > 4$ dB loss) and percentage of test points (percentage) with decreased retinal sensitivity compared with age-matched normal values outside atrophy are shown for group 1.

number of light stimuli applied to areas of normal FAF compared with areas of increased FAF differed greatly because of marked variation in the extent of GA patches. Stimulus locations in FP in group 2 were 17.6 ± 20.3 in areas of normal FAF and 38.2 ± 24.0 in areas with increased FAF. Because of the differences in the number of test points, the comparison between eyes in group 2 is limited. Areas with increased FAF (52.6% ± 29.7%) showed a higher percentage of stimuli with decreased sensitivity than did areas with normal FAF (38.0% ± 33.0%; $P = 0.023$; Fig. 2B).

DISCUSSION

The results show an overall difference in the number of test points with decreased retinal sensitivity between eyes with increased FAF and eyes with normal FAF in the junctional zone of atrophy in patients with GA due to AMD. The average percentage of decreased light stimuli was significantly higher in eyes with increased FAF signals. The comparison of areas with increased FAF and areas with normal FAF within eyes (group 2) also resulted in a higher percentage of locations with

TABLE 2. Summary of Demographics and Results in Eyes with Increased FAF in the Junctional Zone of Atrophy in Group 2

								Test Points with Increased FAF			Test Points with Normal FAF		
No.	Eye	Age	VA	GA Area	*n*	*n* > 4 dB Loss	Percentage	*n* Total	*n* > 4 dB Loss	Percentage	*n* Total	*n* > 4 dB Loss	Percentage
1	R	81	10/25	7.57	55	14	25.5	17	6	35.3	38	8	21.1
2	L	88	10/50	6.91	32	10	31.3	3	3	100.0	29	7	24.1
3	L	81	10/32	10.20	94	81	86.2	15	15	100.0	79	66	83.5
4	L	73	10/50	26.83	47	16	34.0	16	7	43.8	31	9	29.0
5	R	68	10/50	4.09	56	2	3.6	56	2	3.6	0	0	NA
6	L	77	5/160	13.54	41	28	68.3	24	22	91.7	17	6	35.3
7	L	68	5/63	7.94	60	23	38.3	37	22	59.5	23	1	4.3
8	L	63	10/12	13.94	71	43	60.6	20	14	70.0	51	29	56.9
9	L	81	10/100	12.52	31	6	19.4	29	5	17.2	2	1	50.0
10	R	87	5/125	5.73	60	54	90.0	60	48	80.0	12	8	66.7
11	R	83	10/20	4.81	55	46	83.6	54	44	81.5	3	2	66.7
12	R	86	10/32	7.28	25	19	76.0	15	7	46.7	13	12	92.3
13	L	81	10/20	6.83	63	62	98.4	63	62	98.4	0	0	NA
14	L	65	10/63	1.14	65	15	23.1	46	11	23.9	19	4	21.1
15	R	68	10/50	27.84	36	7	19.4	16	7	43.8	20	0	0.0
16	R	74	10/32	14.65	44	11	25.0	44	11	25.0	0	0	NA
17	L	72	10/50	13.08	60	3	5.0	43	3	7.0	17	0	0.0
18	L	71	5/80	NA	50	8	16.0	50	8	16.0	0	0	NA
19	R	71	5/63	10.84	55	34	61.8	55	34	61.8	0	0	NA
20	L	58	10/32	2.62	108	52	48.1	108	52	48.1	0	0	NA
21	L	79	10/25	17.21	50	39	78.0	45	33	73.3	6	6	100.0
22	L	81	10/100	24.36	31	14	45.2	21	14	66.7	10	0	0.0
23	R	74	10/63	23.01	42	21	50.0	27	17	63.0	15	4	26.7
24	L	71	10/100	7.29	75	19	25.3	75	19	25.3	0	0	NA
25	L	74	10/25	3.36	69	8	11.6	15	5	33.3	54	3	5.6

Data are as in Table 1, but are for group 2. In addition, total numbers (*n* total) of tested light stimuli over areas with increased and over areas with normal background FAF are separated, and their numbers ($n > 4$ dB loss) and percentages (percentage) of reduced test points are illustrated.

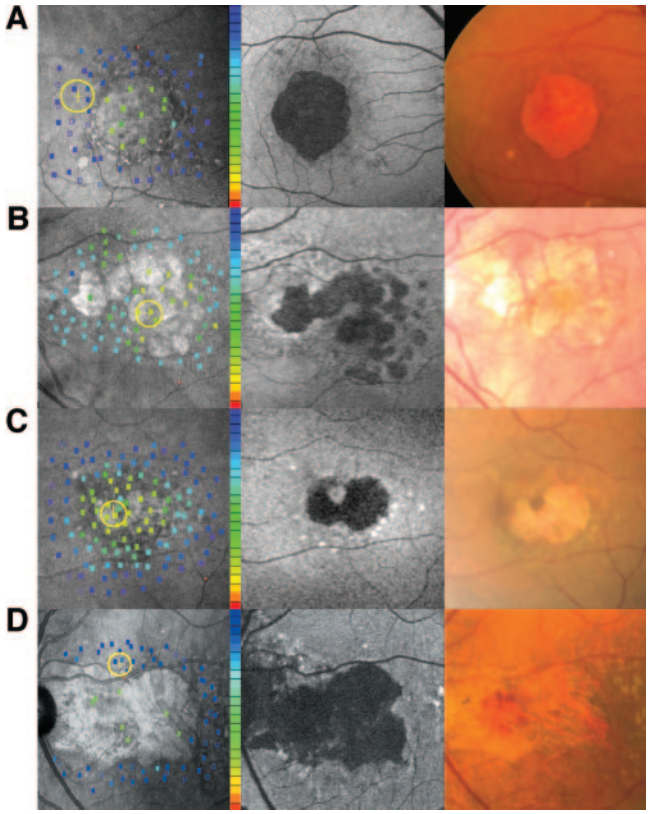

FIGURE 1. For four eyes from groups 1 (**A**) and 2 (**B–D**), the (*left*) FP results, the (*middle*) FAF image, and the (*right*) fundus photograph are shown. The FP image shows Goldmann III size test point results. *Colored rectangles*: the loss of local retinal sensitivity in comparison with age-adjusted normal values. With the help of the scale on the *right* of the FP image (in 1-dB steps), the individual values can be read. *Blue rectangles*: points with small reductions; *red points*: a decrease of 23 dB. A decrease of >4 dB was classified as a decrease in retinal sensitivity. *Open rectangles*: values better than normal. *Yellow cross surrounded by a circle*: the center of fixation. On the FAF image situated in the middle, atrophic areas, the optic disc and retinal vessel show a reduced FAF signal and appear dark. Areas of increased FAF around the atrophy can be distinguished from the normal background signal by an elevated signal intensity. (**A**) Right eye of a 67-year-old male patient with a visual acuity of 10/63. Fixation is located on the temporal border of the single atrophic area. There is no increased FAF outside the atrophic area (group 1). FP shows extensive decreased retinal sensitivity on the atrophy, with near-to-normal values in its vicinity according to the color-coded scale on the *right*, with 0 dB on top and 23 dB at the bottom. (**B**) Left eye of an 81-year-old female patient with visual acuity of 10/20 (group 2). The atrophy encompasses several small confluent atrophic areas. Fixation remains inside the atrophy in the residual fovea. A fine granular pattern of increased FAF is observed in the junctional zone of the atrophy, which is clearly visible toward the disc. FP shows general threshold reduction on, between, and around the atrophy. (**C**) Left eye of a 59-year-old female patient with visual acuity of 10/32 (group 2). Fixation still remained in the center of the single heterogeneous atrophic area. Several focal areas of increased FAF were situated in the junctional zone. FP shows extensive decreased retinal sensitivity on and around the atrophic area, with nearly normal values toward the periphery. (**D**) Left eye of a 72-year-old male patient with visual acuity of 10/50 (group 2). This patient had a large atrophic area that reached the nasal margin of the optical disc. Fixation was above the atrophy. There was a diffuse branching pattern of increased FAF around the atrophy. FP shows extensive decreased retinal sensitivity on the atrophy but, with exception of one target (*light blue* spot in the inferior area), there was only loss of up to 4 dB, or better than average values, at the borders.

sensitivity loss over areas with increased FAF. These results suggest that excessive LF accumulation in RPE cells surrounding areas of GA has a functional correlate.

Controversial views have emerged regarding the pathophysiological relevance of accumulation of LF in the RPE.[8–12] Although there is an age-related increase in LF in postmitotic RPE cells, accumulation is accelerated in various monogenetic retinal diseases, including Best disease and Stargardt disease, and in complex degenerative diseases, such as AMD. LF accumulation appears to be a common pathogenetic pathway in various etiologically heterogeneous hereditary and complex retinal degenerations.

Recent experimental studies have addressed possible molecular mechanisms of interference of LF with normal cell function. Certain compounds of LF may exert toxic effects. A2-E, a dominant fluorophore of LF, is capable of impairing vital RPE lysosomal functions by various mechanisms.[14,31,32] These include a striking inhibition of lysosomal degradation by increasing intralyosomal pH and detergent effects on the lysosomal and other organelle membranes above critical levels.

Normal photoreceptor function is dependent on normal RPE cell function for its contribution to the visual cycle and, in particular, for the constant phagocytosis of shed distal outer segment stacks, a process that generates photoreceptor cell renewal. If LF inhibits degradative metabolism, it would be assumed that the rate of phagocytosis of photoreceptor outer segment (POS) discs is impaired. A negative-feedback mechanism had been proposed, wherein cells with LF-loaded secondary lysosomes would phagocytose less shed POS.[32] If these RPE cells were incapable of clearing obsolete tips of POS to a sufficient degree, it would be assumed that abnormal photoreceptor function would result. These may be the mechanisms or account for the association of increased LF in RPE and impaired photoreceptor function.

GA is a cause of severe irreversible visual loss in patients with AMD, with gradual progression and enlargement of atrophy over time.[4–7] With confocal SLO-FAF imaging, various patterns of increased FAF have been identified in the junctional zone outside the atrophic patches. These areas of increased FAF may precede the enlargement of preexisting atrophy and the development of new atrophic patches over time.[23]

FP is a precise method for the delineation of smaller scotomata in the macular area as well as determining parameters of visual function apart from visual acuity.[25–27] Additional information can be derived from the locus and the behavior of fixation and its stability.[33–35]

In this study, a significant difference of reduction in retinal sensitivity in comparison to age-matched normal values was shown between eyes with normal FAF in the junctional zone of atrophy (group 1) and eyes with different degrees of increased FAF patterns (group 2). The overall average reduction was greater when areas with increased FAF were detected in the eyes (44.9% vs. 20.7% of the tested light stimuli) and was statistically significant in a multiple-regression model in which the size of total GA area and group affiliation are the covariates. The heterogeneity of the size of the total GA area of the studied eyes is caused by the great variety in the extension of atrophy in patients with GA. Further explanations for the statistical differences of total atrophy between both groups are the inability to measure the size of atrophy in three studied eyes and our clinical observation that it is very unlikely that no increased local FAF would be found around atrophies of greater extension in patients with advanced GA due to AMD. Scholl et al.[36] recently also showed a sensitivity loss in eyes with increased FAF. However, they used a different method for functional evaluation (i.e., fine-matrix mapping) and did not assess the function in the junctional zone of GA . They found that scotopic sensitivity loss exceeds photopic sensitivity loss. SLO-

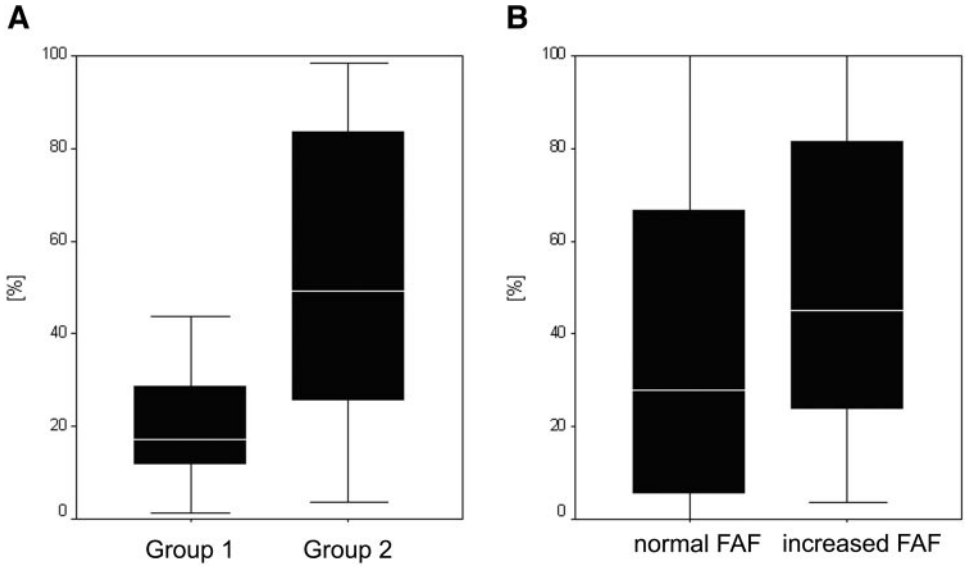

FIGURE 2. (**A**) Box plot showing the percentage of stimulus locations outside GA with reduced light sensitivity to all tested locations outside GA for groups 1 and 2. In a multiple-regression model with retinal sensitivity as the outcome variable and group affiliation and GA area as covariates, the differences were statistically significant ($P = 0.0063$). (**B**) Box plot showing the percentage of stimulus locations outside GA with reduced light sensitivity over areas with increased and normal FAF within group 2. The differences between the two subgroups were statistically significant ($P = 0.023$, Wilcoxon signed rank test).

based fundus perimetry, as applied in our study, does not allow for differentiation between photopic or scotopic photoreceptor responses.

In the functional analysis of areas with different degrees of increased FAF compared with areas with normal FAF in the junctional zone within eyes (group 2), the overall average reduction was greater in areas with different degrees of increased FAF (52.6% vs. 38.0%). However, various limitations have to be considered when interpreting these observations. Because of individual differences in size, location, and number of atrophic areas and the resultant fixation points between the eyes, no standardized testing grid could be applied. Although test points were applied as close to the edge of the GA area in group 1 as in group 2, the number and location of the tested light stimuli differ between tested eyes. The eyes had marked interindividual differences with regard to the distribution of areas with increased FAF. Whereas in some eyes an elevated FAF was detected in the whole junctional zone of atrophy, in other eyes, only a small branching or focal pattern of increased FAF was present (Schmitz-Valckenberg S, et al. *IOVS* 2002;43: ARVO E-Abstract 2518).[22,23] These morphologic differences seen on FAF imaging resulted in different relations between retinal test points in areas with increased FAF compared with test points in areas with normal FAF, limiting the analysis and statistical evaluation of the data.

Sunness et al.[37] described no significant sensitivity between drusen and nondrusen areas within patients and suggested that drusen represent a diffuse disease. Using FAF and comparing

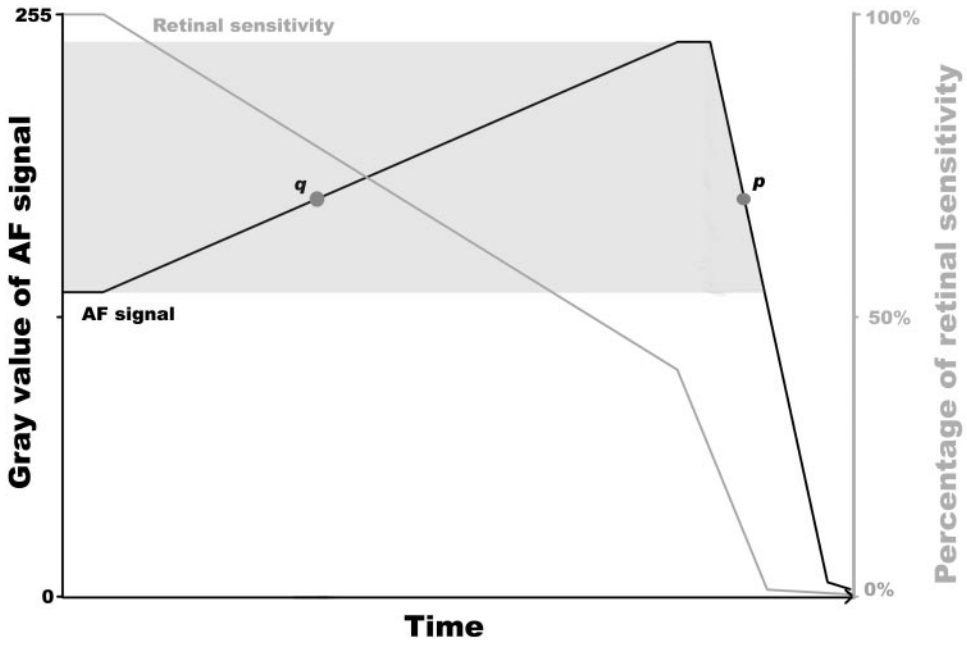

FIGURE 3. Theoretical model for the relationship between FAF signal and retinal sensitivity over time in the junctional zone of GA. The *left* vertical axis shows the gray value of the detected FAF signal (0 = black, 255 = white), whereas the retinal function is illustrated by the right vertical axis (0% = no function, 100% = normal function). The time course of these two different parameters is drawn from *left* to *right* for the FAF signal by the *black* graph and for the retinal function by the *gray* graph. At the beginning, the normal background FAF signal and normal visual function are measured. With increasing LF accumulation, the FAF signal, and consequently its gray value, continuously increase while a reduction in retinal sensitivity occurs. When the LF accumulation reaches a critical level (its maximum), which would cause an FAF signal close to the maximum gray value, RPE cell death (i.e., atrophy) occurs, with a concomitant loss of LF granules and, thus, FAF signal. When the RPE cells become atrophic, retinal sensitivity drops to 0%. Points p and q on the FAF signal graph have the same gray value as the FAF signal, but they do not represent the same time point in the disease process or the same retinal function. It would not be possible by a single FAF image to say whether the RPE cells are in the process of increasing LF accumulation (the ascending part of the FAF signal graph) or whether the cells had already reached their maximum LF levels and would be in the process of development of atrophy (the descending part of the FAF signal graph).

areas with normal versus increased FAF, we showed in our study a functional correlate of decreased retinal sensitivity in areas with increased FAF. However, we could not observe a clear pattern of different degrees of elevated FAF and reduction of retinal sensitivity. Within eyes, different degrees of impaired retinal sensitivity were observed in retinal loci that showed similar FAF signals, whereas a similar decrease in retinal sensitivity was seen in retinal loci that had different FAF signals. This may indicate a more complex relationship between LF accumulation in the RPE, detected levels of FAF, and measured reduction of retinal dysfunction. Various explanations could be considered. First, increased FAF and LF accumulation may represent an epiphenomenon and not a causative factor (i.e., excessive LF accumulation may be an expression of RPE cell dysfunction rather than being a cause of it). Second, a reproducible quantitative measurement of the FAF signal (gray levels) over localized retinal areas is not possible with the instrument used in our study. This is largely because media opacities—with lens opacities being the most important factor—are associated with different degrees of absorption of light in the wavelength range used for excitation and emission in FAF imaging. In this study, we qualitatively distinguished localized retinal areas with normal background from areas with increased FAF, but we could not perform a precise quantitative comparison of the FAF signal and the function measured by FP.

Third, the time course may play a role that has not been taken into account in this cross-sectional study. To date, the time frame from detectable increased FAF signal with impaired photoreceptor function to developing atrophy along with an absolute scotoma has not been investigated. A possible relationship over time between increased FAF, visual function, and atrophy of the RPE is illustrated in a theoretical model in Figure 3. The gray values of the FAF image show only the FAF signals at the time of the examination. Although it can be assumed that any area of increased FAF observed at a certain time had been an area with normal background signal earlier, it may not be possible to determine whether an area with elevated FAF is in the process of increasing accumulation of LF (point *q*) or is in the state of turning from maximum accumulation of LF to development of atrophy (point *p*) (i.e., if the gray value of the area is represented at the ascending or the descending part of the FAF signal graph). Therefore, it is impossible to define the stage of the disease process of a localized area with increased FAF on a single FAF image without having previous or follow-up images.

To evaluate the time course of changes in FAF signals and to study the relationship between different degrees of increased FAF patterns and retinal sensitivity over time, we initiated a longitudinal study with patients with GA using fundus FAF and FP with regular review.

In summary, these findings suggest that elevated FAF outside GA areas is associated with variable degrees of functional impairment of the neurosensory retina. These findings may reflect the relevance of excessive LF accumulations in RPE cells in the context of developing advanced atrophic AMD.

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