Fundus Autofluorescence and Development of Geographic Atrophy in Age-Related Macular Degeneration

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PURPOSE. To describe the development of new and enlargement of preexisting atrophy confined to areas with abnormally high levels of in vivo autofluorescence in eyes with geographic atrophy (GA) associated with age-related macular degeneration (ARMD).

METHODS. The spatial distribution and intensity of fundus autofluorescence as well as the spread of GA and occurrence of new GA was recorded over a period of 3 years in three patients with ARMD using a confocal scanning laser ophthalmoscope.

RESULTS. A diffuse irregular increased autofluorescence at the posterior pole was recorded at baseline in the presence of unifocal or multifocal patches of geographic atrophy. Within these areas of elevated autofluorescence, new atrophic areas developed, and existing patches of atrophy enlarged during the review period, whereas this was not observed in areas with normal background autofluorescence. The total area of abnormal autofluorescence also showed enlargement over time.

CONCLUSIONS. These preliminary findings suggest that areas of increased autofluorescence precede the development and enlargement of outer retinal atrophy in eyes with ARMD. Because the dominant fluorophores of fundus autofluorescence are part of lipofuscin granules of RPE cells, the observations indicate that excessive RPE lipofuscin accumulation may be of significance in the pathogenesis of GA associated with ARMD. With GA being a major cause for severe visual loss in ARMD, in vivo fundus autofluorescence recording over time may allow identification of prognostic determinants and may give important clues to the understanding of mechanisms of disease.

In human postmitotic retinal pigment epithelial (RPE) cells, lipofuscin accumulates with age within the lysosomal compartment.1-3 It is mainly derived from the chemically modified residues of incompletely digested photoreceptor outer segment discs.3,4 Controversy exists with regard to effects of lipofuscin on RPE cell function and its relevance to retinal degeneration.5-7 In the past, lipofuscin accumulation has been largely studied in vitro using fluorescence microscopic techniques.1,2,8 Fundus spectrophotometric studies by Delori et al.9,10 have shown that fundus autofluorescence in vivo is mainly derived from RPE lipofuscin. With the advent of scanning laser ophthalmoscopy, it is now possible to image fundus autofluorescence and its spatial distribution over large retinal areas in vivo.11-14 ARMD is the most common cause of irreversible loss of central vision and legal blindness in developed countries.15-17 Besides choroidal neovascularization and pigment epithelial detachment, geographic atrophy (GA) of the RPE is a frequent cause of severe visual loss in patients with ARMD.18-20 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 at present.

Abnormal autofluorescence patterns in the junctional zone of GA have recently been demonstrated using confocal scanning laser ophthalmoscopy in patients with ARMD.12,21 However, the implications of abnormal autofluorescence outside areas of atrophy in the subsequent course of the disease has not been elucidated to date. To evaluate the significance of excessive lipofuscin accumulation surrounding areas of GA associated with ARMD, we obtained serial fundus autofluorescence images and recorded changes in preexisting and occurrence of new atrophic areas in a prospective longitudinal study.

METHODS

Fundus autofluorescence was recorded using a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph, HRA; Heidelberg Engineering, Heidelberg, Germany), the optical and technical principles of which have been described previously.21,22 In brief, two scanning mirrors provide the horizontal and vertical scanning directions. The illumination beam has a diameter of 3 mm, and the full aperture of the dilated or undilated eye is used to collect light from the posterior pole. The rectangular field of view can be adjusted to 10° × 10°, 20° × 20°, or 30° × 30°. The confocal detection unit uses a small pinhole aperture (400 μm) to suppress light originating outside the focal plane. Thereby, image contrast is enhanced compared with nonconfocal images. The optics of the instrument allow ametropia compensation between −12 and +12 D.

For fundus autofluorescence imaging, an argon blue laser (488 nm) is used for excitation. Emitted light is detected above 500 nm (barrier filter). With an interference filter, the green share of the argon laser is used for excitation. The above-mentioned cutoff filter suppresses blue argon excitation light at 488 nm by a factor of 10⁻⁶. Consequently, it is assumed that reflectance signals do not contribute to the autofluorescence image obtained from the posterior pole of the examined eye. To amplify the autofluorescence signal, several images

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can be aligned, and a mean image can be calculated after detection and correction of eye movements, by using image analysis software.\textsuperscript{21}

Maximal retinal irradiance using the HRA is approximately 2 mW/cm\textsuperscript{2} for a 10° × 10° frame and is therefore well below the limits established by the American National Standards Institute (ANSI) and other international standards (ANSI Z136.1; 1995). Images are immediately digitized and processed using a flexible frame processor and subsequently displayed on a computer screen. The frame grabber can digitize image frames at a programmable rate of up to 20 frames per second. Each frame contains 256 (or 512) pixels in vertical and 256 (or 512) pixels in horizontal direction. Images are digitized with 256 gray levels; thus, each single image contains 64 (or 256) kilobytes. The digital images are saved on hard disc for further analysis and processing.

The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee by the University of Heidelberg.

Before examination, the pupil of the study eye was dilated to a diameter of at least 6.5 mm with 1% tropicamide. Fundus photographs were obtained and best corrected central visual acuity was determined using Early Treatment Diabetic Retinopathy Study (ETDRS) charts.

Abnormal autofluorescence was defined either as an increased or decreased fundus autofluorescence signal compared with the autofluorescence outside such lesions, the latter being referred to as normal fundus autofluorescence. Autofluorescence images in normal eyes have been published previously.\textsuperscript{11,13} They show an even distribution in autofluorescence, with a typical decrease in intensity in the macular area due to absorption of the yellow pigment in the sensory retina and lower lipofuscin levels in central RPE cells. There is also a lower signal along large retinal vessels (absorption) and at the optic disc (absence of autofluorescent material). It is known from in vivo and in vitro studies that with age, a continuous accumulation of autofluorescent material occurs at the level of the retinal pigment epithelium.\textsuperscript{5,6} At the same time, variation with age also increases. However, there is currently no means to reliably distinguish normal age-related levels from pathologic accumulations if there are no localized changes.

Important for the distinction between normal and abnormal autofluorescence in the images obtained with the scanning laser ophthalmoscope is the spatial distribution of autofluorescence signals in comparison with normal eyes. Areas of geographic atrophy typically show a markedly reduced degree of autofluorescence, as previously shown.\textsuperscript{12,21} Areas of abnormally high autofluorescence, as delineated herein, usually have a stippled pattern and do not show an evenly distributed elevated autofluorescence intensity. Such areas may encompass small patches with normal or only minimally elevated autofluorescence. However, entire areas that encompass an abnormal autofluorescence including irregular elevated autofluorescence signals and patches of normal-appearing regions can be distinguished from areas with normal background autofluorescence, in that the latter show an even and lower autofluorescence pattern.

Various patterns of abnormal autofluorescence outside geographic atrophic patches in association with ARMD have been described previously.\textsuperscript{21} The most common type is a circumferential small band with increased autofluorescence. Less common is a diffusely irregular autofluorescence outside the areas of atrophy, the shape of which shows no correspondence with the encompassed contours of the atrophic areas. Finally, in a subset of eyes, focal small patches of elevated autofluorescence only are seen at the margin of the atrophic zone.

Planimetric measurements were performed by encircling the area of interest (i.e., abnormal autofluorescence or atrophy) with a mouse-driven arrow and calculating the area, using image analysis software. If the peripheral margins of the area with abnormally high autofluorescence were not precisely outlined or were too irregularly shaped, the area of a convex hull was measured.\textsuperscript{22} This encompassed the entire area of abnormal autofluorescence connecting the outer peripheral elevated signals.

Fundus autofluorescence images in a cross-sectional survey conducted at the Department of Ophthalmology, University of Heidelberg, so far have been obtained in 142 patients (mean age, 75.9 ± 7.3 years) with GA associated with ARMD. Of 142 patients in whom autofluorescence images were recorded, image quality was sufficient in 102 patients (71.8%) to allow classification into the following categories: no abnormal autofluorescence, diffuse, band, or focal elevated autofluorescence outside the area of atrophy.\textsuperscript{21} Of 102 patients, 15 (14.7%) had no abnormal autofluorescence outside the atrophic area. Of 87 patients with abnormal autofluorescence 64 (73.6%) had a small continuous band, 14 (16.1%) had a diffuse type, and 9 (10.3%) had a focal type of elevated autofluorescence in the junctional zone. Three patients with the diffuse type reported herein had sufficient image quality both at baseline and at subsequent examinations to allow a meaningful analysis over a review period of 3 years in this pilot study. Serial examinations included visual acuity, fundus biomicroscopy, fundus photography, and fundus autofluorescence imaging using the HRA.

**RESULTS**

In all examined eyes absent autofluorescence corresponded spatially with areas of GA visualized with funduscopy and documented with fundus photographs. There was no obvious incipient atrophy on funduscopy as previously defined by Sarks.\textsuperscript{20}

Fundus autofluorescence imaging was not performed at baseline in 10 of 142 patients (7.0%) due to restricted cooperation or corneal opacities. At baseline the area of abnormal autofluorescence encompassed the entire area of GA in 56 (55.0%) of 102 examined eyes; in 40 (39.2%) of 102 examined eyes, the abnormal autofluorescence encompassed only the junctional zone of variable peripheral extension; areas with normal-appearing regions could be distinguished from areas with abnormal autofluorescence in 10 (9.8%) of 102 examined eyes (Figs. 2, 3, 4).

**Patient 1**

In the left eye of a 55-year-old male patient with ARMD a large kidney-shaped area of GA was noted initially (Fig. 2A). Best corrected visual acuity was 20/32 OS. Funduscopy showed minor pigmentary changes outside the area of atrophy and a few small drusen, the distribution of which did not correspond to the areas with abnormal autofluorescence. On autofluorescence imaging the HRA was used to examine the left eye of this patient. Of 102 patients, 15 (14.7%) had no abnormal autofluorescence outside the atrophic area. Of 87 patients with abnormal autofluorescence 64 (73.6%) had a small continuous band, 14 (16.1%) had a diffuse type, and 9 (10.3%) had a focal type of elevated autofluorescence in the junctional zone. Three patients with the diffuse type reported herein had sufficient image quality both at baseline and at subsequent examinations to allow a meaningful analysis over a review period of 3 years in this pilot study. Serial examinations included visual acuity, fundus biomicroscopy, fundus photography, and fundus autofluorescence imaging using the HRA.

**FIGURE 1.** Fundus photographs from all eyes at baseline (A, patient 1; B, patient 2; C, patient 3). There was no obvious incipient atrophy on funduscopy as previously defined by Sarks.\textsuperscript{20}
cence imaging, there was an increased signal surrounding the atrophic patch that is broader on the nasal side compared with the superior, temporal, and inferior border. With time, the whole atrophic area increased from 14.3 to 16.7 mm$^2$ at 1 year, to 18.3 mm$^2$ at 2 years, and to 20.7 mm$^2$ at 3 years (Figs. 2A through 2D). At the nasal junctional zone, five new atrophic patches occurred, whereas there was a single atrophic patch noted at the temporal margin during the most recent visit.

**Patient 2**

A 65-year-old female patient initially had several small patches of GA in the right eye. Visual acuity was 20/32. There were scattered nonconfluent drusen visible at the posterior pole as well as minor pigmentary alterations. Autofluorescence was absent in the visible areas of GA (Fig. 3A). On fundus autofluorescence, there was a spotty autofluorescence signal that encompassed all the small atrophic patches when compared with the normal background autofluorescent signal peripheral to the posterior pole. One year after initial examination, there were more small patches of atrophy as well as enlargement of preexisting spots of atrophy noted in the area that showed increased autofluorescence (Fig. 3B). Further spread of atrophy was noted 1 year thereafter—i.e., 2 and 3 years after the initial visit (Figs. 3C, 3D). No new atrophy occurred in areas where autofluorescence was normal at baseline. The total area of atrophy enlarged from 1.35 to 4.29 mm$^2$ during the review period.

Planimetric measurement of the total area of abnormal autofluorescence showed 27.4 mm$^2$ at baseline and 31.1 mm$^2$ after 3 years. This is equivalent to a 15.5% change in size of the area with abnormal autofluorescence.

**Patient 3**

At baseline examination of the left eye of a 65-year-old male patient, a large central area of GA was noted, and several small patches of atrophy were seen superior and nasally superior to the large area (Fig. 3A). Outside the atrophic patch, a few drusen were noted superior and temporal to the large atrophic patch. Visual acuity at that time was 20/160 OS. Fundus autofluorescence showed an elevated autofluorescence signal in the junctional zone with a small band at the temporal and inferior margin and a broader zone in the nasal and superior junctional zone that encompassed the small atrophic patches (Fig. 4A).

During the subsequent yearly reviews (Figs. 4B, 4C, 4D), the small preexisting atrophic patches increased in size, and new patches of atrophy occurred. The atrophic area increased in size from 11.1 to 11.8 mm$^2$ at 1 year, to 12.9 mm$^2$ at 2 years, and 13.8 mm$^2$ at 3 years. All new areas of GA developed in areas of abnormally high fundus autofluorescence when compared with background. Some of the patches became coalescent over time. Visual acuity remained unchanged during the review.

Planimetric measurement of the total area of abnormal autofluorescence demonstrated an enlargement in size from 27.9 mm$^2$ at baseline to 31.1 mm$^2$ after 3 years. This is equivalent to a 11.5% change with respect to the area of abnormal autofluorescence.
brane. However, the pathophysiological mechanisms of lesions are associated with changes in the outer retina and the atrophic process, involving the RPE, the neurosensory background fluorescence.

Occurrence of atrophy was not noted in areas with normal abnormally high levels of fundus autofluorescence at baseline. Serial fundus autofluorescence examinations in patients with ARMD. There is evidence to suggest that such lesions are associated with changes in the outer retina and with accumulation of debris at the level of Bruch’s membrane. However, the pathophysiological mechanisms of the atrophic process, involving the RPE, the neurosensory autofluorescence compared with a 24.6% enlargement of the atrophic area.

DISCUSSION

Serial fundus autofluorescence examinations in patients with GA in this pilot study disclosed the occurrence of new atrophic patches and spread of preexisting atrophy solely in areas with abnormally high levels of fundus autofluorescence at baseline. Occurrence of atrophy was not noted in areas with normal background fluorescence.

Fundus spectrophotometric studies with spectral analysis indicated that RPE lipofuscin contains the dominant fluorophores of fundus autofluorescence. The longitudinal variations observed in the present study in a small number of patients indicate an association of excessive RPE lipofuscin accumulation and development of GA (i.e., cell death at the level of the RPE, outer neurosensory retina, and choriocapillaris). Two principal explanations may be considered regarding the nature of association: Lipofuscin may play a direct pathogenetic role by causing RPE dysfunction with subsequent deleterious effects. Alternatively, excessive accumulation of lipofuscin is an expression of RPE cell dysfunction rather than a cause of it.

GA is a cause of severe irreversible visual loss in patients with ARMD. There is evidence to suggest that such lesions are associated with changes in the outer retina and with accumulation of debris at the level of Bruch’s membrane. However, the pathophysiological mechanisms of the atrophic process, involving the RPE, the neurosensory retina, and the choriocapillaris, are poorly understood. In a cross-sectional study on eyes with ARMD, increased accumulations of autofluorescent material at the level of the RPE were most frequently been observed in eyes with GA when compared with eyes with other ARMD manifestations. Rückmann et al. initially described the presence of elevated autofluorescence surrounding atrophic areas in eyes with ARMD. In addition, various patterns of changes in fundus autofluorescence may occur in the junctional zone of GA reflecting heterogeneity of the underlying disease process.

In vitro ultrastructural studies of eyes with geographic atrophy have been performed in the past. Lipofuscin and melanolipofuscin-filled RPE-cells were observed in the junctional zone between the atrophic and the normal retina. As the atrophic region was approached, the RPE became increasingly abnormal in shape, and cell loss was evident. Closer to the edge of the atrophic area, large hyperpigmented RPE cells were being shed into the subretinal space. Many contained large membrane-bound bodies filled with fused lipofuscin and melanolipofuscin granules. It was assumed that the accumulation of lipofuscin and the subsequent increased autofluorescence granules in these cells is not only the result of autophagy and outer segment phagocytosis but also of engulfment of cellular debris including spent RPE cells.

In contrast to histologic findings, examination with the confocal scanning laser ophthalmoscope gives information on the spatial distribution of variations in lipofuscin-mediated fundus autofluorescence and allows for examination in vivo over time. The fundus autofluorescence findings in patients with GA show similarities with the in vitro observations. Lipofuscin-laden cells in the junctional zone may correspond to the band of increased autofluorescence surrounding the atrophic patch. However, the size of the junctional zone with increased autofluorescence shows marked interindividual variation with regard to its peripheral extension in vivo. In addition, it is usually broader than the hyperpigmented rim seen ophthalmoscopically.

In contrast to histologic findings reported so far, our findings indicate that the so-called junctional zone with abnormal RPE may extend far beyond a small band at the margin of the atrophic patch. It may be speculated either that eyes with such widespread change have not yet been examined histologically or that they have not been reported.

Diffusely increased autofluorescence at the posterior pole associated with GA may reflect a large area of incipient atrophy. The term incipient atrophy was initially introduced by Green and Enger and Sarks and is characterized by semisolid drusen (i.e., small dot-like drusen, 25–50 μm in size) and a microreticular pigment pattern. Although there were few drusen and minor pigmented irregularities outside the atrophic patches in the eyes presented herein, these changes were not identical with Sarks’s description of incipient atrophy. Some of the drusen were larger, and, more important, in some areas with elevated autofluorescence, there was no visible alteration on funduscopy or color photographs at all. Therefore, fundus autofluorescence imaging obviously provides additional information over and above conventional fundus photographs.

Based on these preliminary observations, areas of incipient atrophy may be more accurately delineated with scanning laser ophthalmoscope autofluorescence imaging than with conventional funduscopy or fundus photography. The eyes reported in this study had several patches of atrophy indicating that cell death did not occur exclusively at the junctional zone of a single atrophic patch but at many sites at the posterior pole.
simultaneously in the presence of diffuse change. Multifocal patches of atrophy have been shown to increase in size with time and to coalesce, resulting in a large patch of atrophy as the endstage of the disease process. Eyes with such diffuse increased autofluorescence may be at particularly high risk for the development of large scotomas and loss of central vision with foveal involvement.

Several previous studies have described the progression of GA over time. In a retrospective study, Schatz and McDonald found a rate of spread of geographic atrophy growth of 15 to 375 μm (average, 139 μm) per year, whereby smaller areas tended to grow slower than larger atrophic areas. In a prospective study of the natural history of the progression of GA, Sunness et al. recently demonstrated a mean enlargement of the total GA area of 2.2 disc area (DA) per 2 years. In this study the amount of enlargement increased with increasing baseline total atrophy up to 5 DA of baseline atrophy, and, interestingly, leveled off at more than 5 DA. Whereas Sarks hypothesized that atrophy stops enlarging once all incipient atrophy characterized by areas of pigment epithelial attenuation and focal hyperpigmentation has become involved, Sunness et al. showed enlargement of atrophy at all baseline atrophy levels. However, it can only be speculated how far the atrophy would eventually spread if the affected patient could be observed for a period far beyond normal life span.

We observed a spread both of the areas of atrophy and of the total areas of abnormal autofluorescence over time. It may be speculated that atrophy does not grow beyond the area of abnormal autofluorescence and that the area therefore reflects the maximal extent of atrophy during the subsequent clinical course. This observation may also reflect the common restriction of ARM to the central retinal area. Autofluorescence appearance was normal in all eyes studied peripheral to the area of abnormally high autofluorescence, and in none of the eyes studied did GA occur in those areas. If an increased lipofuscin content precedes cell death, it may be speculated that the spread of the atrophic area may be greater and faster toward areas with more intensely increased autofluorescence. Future long-term in vivo studies using the confocal scanning laser ophthalmoscope should address the question of whether the rapidity of spread of the atrophic area correlates with size and intensity of abnormally high autofluorescence in the junctional zone.

In summary, because increased fundus autofluorescence precedes development of GA, it is assumed that topographic detection of variations in fundus autofluorescence using confocal scanning laser ophthalmoscopy may be of prognostic value and may serve to identify patients at high risk for occurrence or enlargement of absolute scotomas and thus severe visual loss. Furthermore, the findings reflect the pathophysiological significance of excessive lipofuscin accumulation in RPE cells. Based on our observations, we initiated an expanded natural history study including fundus autofluorescence examinations in patients with GA. This may provide important information to the understanding of the mechanism of disease and may be helpful in monitoring the effect of future therapeutic interventions.

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References


