

Fundus Autofluorescence Characteristics of Nascent Geographic Atrophy in Age-Related Macular Degeneration

Zhichao Wu,¹ Chi D. Luu,¹ Lauren N. Ayton,¹ Jonathan K. Goh,¹ Lucia M. Lucci,² William C. Hubbard,² Jill L. Hageman,² Gregory S. Hageman,² and Robyn H. Guymer¹

¹Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Victoria, Australia

²Department of Ophthalmology and Visual Sciences, John A. Moran Eye Center, Center for Translational Medicine, University of Utah, Salt Lake City, Utah, United States

Correspondence: Zhichao Wu, Macular Research Unit, Centre for Eye Research Australia, Level 1, 32 Gisborne Street, East Melbourne, VIC 3002, Australia; wu.z@unimelb.edu.au.

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PURPOSE. We examined the fundus autofluorescence (FAF) characteristics of nascent geographic atrophy (nGA), pathological features preceding the development of drusen-associated atrophy in eyes with age-related macular degeneration (AMD) that can be visualized using high-resolution optical coherence tomography (OCT).

METHODS. Spectral-domain OCT (SD-OCT) and FAF imaging were performed longitudinally in 221 eyes with intermediate AMD (having at least drusen >125 μm), and seven areas that developed drusen-associated atrophy in five eyes were examined and categorized with respect to FAF characteristics. These categories then were used to characterize 49 areas of nGA or drusen-associated atrophy on SD-OCT identified in a cross-sectional study with 230 participants with bilateral intermediate AMD.

RESULTS. Sequential imaging revealed that FAF characteristics in the atrophic areas could be grouped into three categories: predominantly hyperautofluorescent (hyperAF), presence of both hyper- and hypoautofluorescence (mixed AF), or predominantly hypoautofluorescent (hypoAF). In the cross-sectional study, the FAF characteristics were significantly dependent on the type of atrophic area ($P = 0.002$), where areas of nGA appeared most commonly as being mixed AF (63%), while areas of drusen-associated atrophy most commonly as hypoAF (86%).

CONCLUSIONS. Fundus autofluorescence imaging revealed that areas of nGA were most commonly characterized by both hyper- and hypoautofluorescent changes, which differs from areas of drusen-associated atrophy that most often appeared hypoautofluorescent. These findings provide important insights into the FAF characteristics of areas undergoing atrophic changes in eyes still considered to be in the early stages of AMD by current methods, and thus assist in the characterization of disease severity in these early stages.

Keywords: age-related macular degeneration, fundus autofluorescence, geographic atrophy, drusen, spectral-domain optical coherence tomography

The presence of drusen and pigmentary abnormalities characterize the early stages of age-related macular degeneration (AMD), and vision-threatening complications, such as geographic atrophy (GA) and choroidal neovascularization (CNV), can develop in eyes with these features over time. Novel imaging modalities and functional measures have been used to further characterize these early stages,¹⁻⁸ to improve the identification of those at risk of progression and to aid in evaluating emerging novel interventions that seek to prevent or slow such progression.

Fundus autofluorescence (FAF) imaging is one such tool that has been used in AMD due to its ability to noninvasively visualize retinal fluorophores, providing unique insights into the disease state. Fundus autofluorescence imaging is often performed using a short-wavelength excitation light source and the emission of the fluorescence signals using this method has been thought to arise predominantly from lipofuscin and melanolipofuscin accumulated in the RPE,^{9,10} although it is also influenced by the degree of absorption by the overlying retinal photopigments.¹¹ Fundus autofluorescence imaging has been

useful in characterizing disease progression in eyes with GA¹²⁻¹⁴ and has been used to evaluate response to interventions for those eyes.¹⁵⁻¹⁷ In the early stages of AMD, distinct patterns of FAF changes have been described and suggested to be useful at determining the risk of progression.^{18,19}

Clinical studies of eyes with the early stages of AMD have found that areas with FAF changes are associated with decreased visual function²⁰ and disruption of the photoreceptors visualized on spectral-domain optical coherence tomography (SD-OCT).²¹ These changes often colocalize with large drusen and hyperpigmentary changes, but do not always correspond with these features.^{5,18,19,21,22} Recent longitudinal studies have observed that drusen regression also can be characterized by different FAF changes, suggesting that FAF imaging may allow different long-term outcomes to be distinguished.^{23,24}

In seeking to improve the characterization of pathological features in the early stages of AMD, we have also recently used SD-OCT to visualize unique characteristics that portend the development of drusen-associated atrophy. We defined these

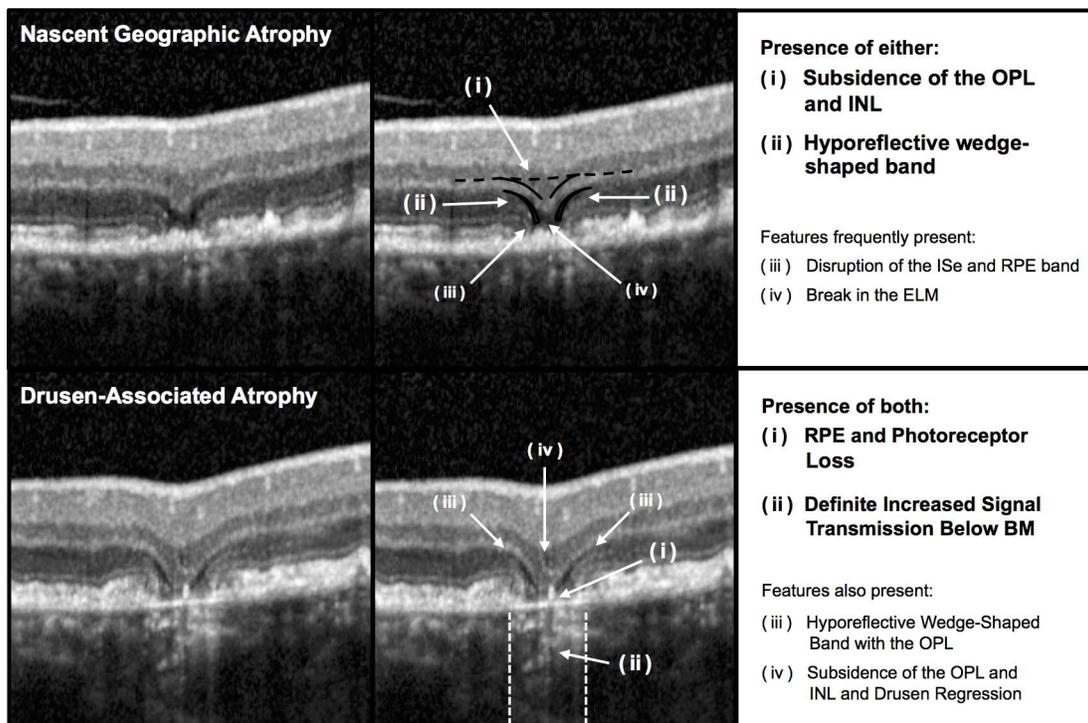


FIGURE 1. Characteristics of nGA and drusen-associated atrophy detected on SD-OCT, shown for an area that was examined longitudinally. The presence of nGA is defined as the presence of the subsidence of the INL and OPL, and/or the presence of a hyporeflective wedge-shaped band (outlined by *black solid lines*) within the limits of the OPL (*top row*). For subsidence of the INL and OPL (*top row*), the *solid black line* outlines the border between the INL and OPL from this scan, while the *dashed black line* outlines its border at the previous visit. Other features also typically present with nGA include a break in the ELM and disruption of the ISe and RPE bands (*top row*). Drusen-associated atrophy detected on SD-OCT is characterized by the loss of the RPE and photoreceptor (ISe) bands, resulting in a definite area of increased signal transmission below BM (*bottom row*).

features as nascent geographic atrophy (nGA), which included the subsidence of the outer plexiform layer (OPL) and inner nuclear layer (INL), and/or the development of a hyporeflective wedge-shaped band within the limits of the OPL, features that were not visible on color fundus photography (CFP).²⁵ We observed that once nGA was detected, drusen-associated atrophy as detected on SD-OCT developed after approximately 1 year on average, and thus hypothesized that these early features provide new information, crucial when attempting to determine the risk of future vision loss. We also observed that areas of nGA had poorer microperimetric sensitivity on average than nonatrophic areas, but were not yet characterized by absolute scotomas.²⁶

We were thus interested in using FAF imaging to characterize areas of nGA to provide further pathological insights into these features present in eyes currently considered to be in the early stages of AMD as determined on CFP. This report describes the FAF characteristics of areas with nGA and drusen-associated atrophy.

METHODS

Participants

The design of this study and participant selection criteria have been described in detail in a previous study.²⁵ Briefly, participants who were involved in Human Ethics or Institutional Review Board-approved AMD research studies that adhered with the Declaration of Helsinki from two different sites, the Centre for Eye Research Australia, Melbourne, and the John A. Moran Eye Center, University of Utah, were retrospec-

tively analyzed in a longitudinal study if they met the inclusion criteria. The inclusion criteria required participants to be over 50 years of age, have more than one drusen >125 μ m as determined on CFP, and best-corrected visual acuity of 20/40 or better in at least one eye. The exclusion criteria for a study eye included any evidence of advanced AMD, including any evidence of GA or CNV at the baseline examination on CFP and SD-OCT (see the "Grading and Image Analysis" section for the definition of GA), any past treatment for CNV, or any other disease that would affect vision or prevent longitudinal evaluation of the pathological features of AMD.

For the longitudinal analysis, only participants whose eyes did not have drusen-associated atrophy detected on SD-OCT at the initial visit and were seen at least three-monthly for a minimum of 12 months were included. Drusen-associated atrophy was defined in a manner similar to previous studies,^{27,28} as the presence of these features visualized on SD-OCT in eyes with drusen (to ensure that it was secondary to AMD): a loss of the RPE and inner-segment ellipsoids (ISe) bands, resulting in increased signal transmission below Bruch's membrane (BM), that also is accompanied by loss of the external limiting membrane (ELM) and outer nuclear layer (ONL) in this area (Fig. 1). These participants were examined longitudinally to determine pathological features preceding the development of drusen-associated atrophy on SD-OCT, and the presence of either or both of these two features—the subsidence of the OPL and INL, and/or a hyporeflective wedge-shaped band within the boundaries of the OPL—were defined as nGA (Fig. 1).²⁵ The FAF images of the participants identified as having developed drusen-associated atrophy detected on SD-OCT then were analyzed to examine the changes in FAF characteristics in these areas over time.

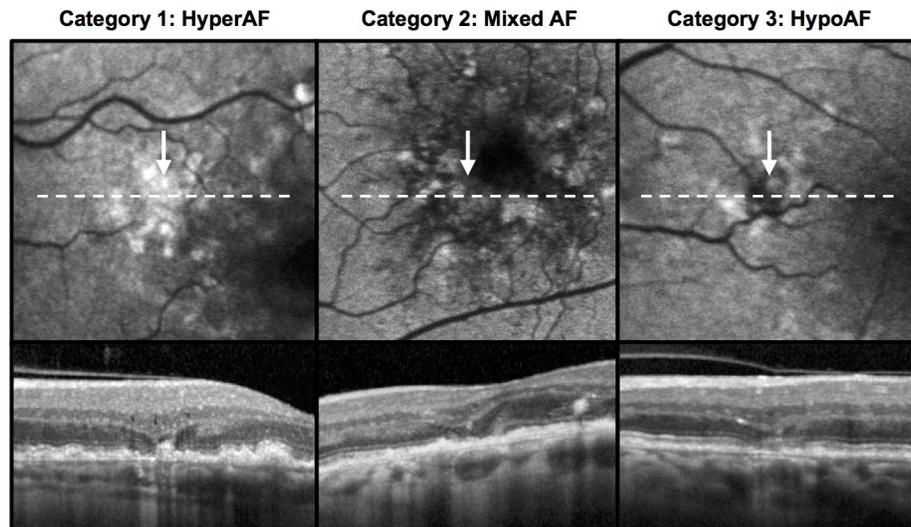


FIGURE 2. Fundus autofluorescence characteristics in atrophic areas (nGA or drusen-associated atrophy detected on SD-OCT) were classified into three different categories: areas that were predominantly hyperautofluorescent (Category 1, HyperAF), areas that were characterized by both hyperautofluorescence and hypoautofluorescence (Category 2, Mixed AF), and areas that were predominantly hypoautofluorescent (Category 3, HypoAF). Note that all SD-OCT scans (*bottom*) show areas of nGA.

Once these features of nGA were determined from the longitudinal study, participants at the two sites with bilateral intermediate AMD (defined as having at least drusen $>125\ \mu\text{m}$ in both eyes), as determined on grading of CFP, who met the same inclusion and exclusion criteria in both eyes, and seen at a minimum of one time-point were examined in a cross-sectional analysis. Images from these participants, including those with bilateral intermediate AMD from the longitudinal cohort, were analyzed to identify eyes with atrophic areas (either nGA or drusen-associated atrophy, detected using SD-OCT) so that the FAF characteristics of these areas could be examined.

Imaging

Near-infrared (NIR) reflectance, FAF, and SD-OCT scans all were performed using the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) and along with CFP, were performed on all participants during all visits. The FAF images were acquired using an excitation wavelength of 488 nm and emitted fluorescence signals were detected between 500 and 700 nm using the confocal scanning laser ophthalmoscope component of this device. A $30^\circ \times 30^\circ$ field was imaged using the high-resolution mode, with a resolution of 1536×1536 pixels. The brightness and contrast adjustments were set automatically by the image acquisition system, and the image was acquired after averaging 25 frames. For SD-OCT, all participants had volume scans performed using 49 B-scans of the central $20^\circ \times 20^\circ$ area, with 25 frames averaged for each B-scan (Melbourne), or 19 horizontal B-scans of the central $15^\circ \times 20^\circ$ area with 10 frames averaged for each B-scan (Utah), using the automatic scan alignment feature for follow-up scans. Note that all participants included in the cross-sectional study had the volume scan protocol ($20^\circ \times 20^\circ$, 25 frames averaged per B-scan) performed, since they were all bilateral intermediate AMD participants seen in Melbourne.

Grading and Image Analysis

The grading of CFP was performed using OptimizePro (Digital Healthcare Image Management System; Digital Healthcare Ltd, Cambridge, UK) by one experienced grader (Melbourne) to determine eligibility for this study. Geographic atrophy was

defined on CFP as a sharply delineated area of RPE hypopigmentation that was larger than $175\ \mu\text{m}$ with the underlying choroidal vessels visible. The grader was masked to SD-OCT, NIR, or FAF images.

The FAF characteristics of the areas that were identified as having developed drusen-associated atrophy detected on SD-OCT in the longitudinal cohort then were examined by four investigators (RHG, CDL, LNA, and ZW), who reached a consensus on grouping these characteristics into three categories (see Results). These three categories then were used when grading the atrophic areas identified in the cross-sectional study by one experienced grader.

Statistical Analyses

To examine whether the FAF characteristics are dependent on the type of atrophic area (nGA or drusen-associated atrophy detected on SD-OCT), a Fisher's exact test of independence was used and the significance level was calculated based on the exact distribution of the test statistic, since the assumptions for the standard asymptomatic calculation of the significance level was not met. All statistical analyses were performed using commercially available statistical software (IBM SPSS Statistics, software version 21; IBM/SPSS, Inc., Chicago, IL).

RESULTS

FAF Changes in the Development of Drusen-Associated Atrophy

From the retrospective longitudinal analysis involving 221 eyes from 181 participants with intermediate AMD (without any drusen-associated atrophy detected on SD-OCT at the initial visit), 20 areas from 16 eyes of 16 participants developed drusen-associated atrophy detected on SD-OCT after an average follow-up of 20 months (range, 8–30 months) from the initial visit. Of these, only seven areas from five eyes of five participants met the inclusion criteria of having FAF imaging of sufficient quality at every visit between the first detection of nGA to the first detection of drusen-associated atrophy detected on SD-OCT. These participants were on average 67

TABLE 1. The FAF Imaging Characteristics at Different Stages in Areas That Subsequently Developed Drusen-Associated Atrophy Detected on SD-OCT

	HyperAF	Mixed AF	HypoAF
Nascent geographic atrophy first detected on SD-OCT	3 (43%)	4 (57%)	-
Drusen-associated atrophy first detected on SD-OCT	-	2 (29%)	5 (71%)

years of age (range, 56–75 years), and nGA was present in all areas before the development of drusen-associated atrophy. The areas of drusen-associated atrophy developed after an average of 11 months following the first detection of nGA (range, 6–20 months).

In these eyes, we observed three predominant characteristics of FAF in the areas that developed drusen-associated atrophy and grouped them into three categories; areas that were predominantly hyperautofluorescent (hyperAF), areas that were characterized by both hyperautofluorescence and hypoautofluorescence (mixed AF), and areas that were predominantly hypoautofluorescent (hypoAF; Fig. 2). Areas of mixed AF were distinguished from areas of predominant hypoautofluorescence with some isoautofluorescence (which were considered to be hypoAF) by taking into account of the regional variation in autofluorescence, such as the decrease in autofluorescence from the parafoveal region to the foveal center. These categories then were used to categorize the FAF characteristics in the atrophic areas of the longitudinal study.

At the first detection of nGA, four of seven areas (57%) were graded as having mixed AF, while the remaining three of seven areas (43%) were graded as hyperAF. When drusen-associated atrophy was detected on SD-OCT, five of seven areas (71%) were graded as hypoAF, while the remaining two of seven areas (29%) were graded as mixed AF; these features are summarized in Table 1.

Sequential imaging on FAF revealed a general decrease in the amount of overlying hyperAF and increase in the appearance of the hypoAF between the first detection of nGA to the development of drusen-associated atrophy in all areas that subsequently had drusen-associated atrophy in this study; an example of this is shown in Figure 3 and Supplementary Video S1.

Cross-sectional Evaluation of Nascent Geographic Atrophy on Fundus Autofluorescence

In the cross-sectional analysis involving 230 participants (460 eyes) with intermediate AMD, a total of 49 areas of nGA were detected in 43 eyes of 37 (16%) participants and 14 areas of drusen-associated atrophy were detected in 11 eyes of nine (4%) participants (5 of the 11 eyes also had nGA).

In areas where nGA were present, 4 (8%), 31 (63%), and 14 (29%) were graded as hyperAF, mixed AF, and hypoAF, respectively. In areas where drusen-associated atrophy was present, 2 (14%) and 12 (86%) were graded as being mixed AF and hypoAF, respectively (Table 2). The Fisher's exact test confirmed that the FAF characteristics were dependent on the type of atrophic area ($P = 0.001$), and areas of nGA appeared most commonly as being mixed AF, while areas of drusen-associated atrophy most commonly as hypoAF.

DISCUSSION

This study sought to describe the FAF characteristics in areas with nGA, features that we have recently described on SD-OCT

that precede the development of drusen-associated atrophy. These unique features of nGA, including the subsidence of the INL and OPL, and the presence of a hyporeflective wedge-shaped band within the limits of the OPL, were often (but not always) accompanied by changes to the RPE and photoreceptors as visualized on SD-OCT.²⁵ On the other hand, areas of drusen-associated atrophy were always defined by a loss of the RPE and photoreceptors on SD-OCT,^{25,27,28} and we thus sought to examine the FAF characteristics in these atrophic areas.

In the cross-sectional analysis of this study, areas of drusen-associated atrophy detected on SD-OCT (but not detected and graded as GA on CFP) most often appeared as areas of hypoAF on FAF. These hypoAF changes exhibit a similar appearance to those that occur in areas of later-stage GA (visible on CFP), where they have been found to be associated with SD-OCT changes, including RPE and photoreceptor loss, and increased signal transmission below BM.^{29–31}

Areas of nGA most often were characterized on FAF by the presence of both hyperAF and hypoAF changes (mixed AF). In areas of GA, its enlargement has been reported to be associated with the presence of hyperAF at its borders.^{32,33} However, a recent histological study showed that the hyperAF often seen at the border of GA represents advanced RPE alterations due to vertically superimposed cells and cellular fragments (rather than early RPE changes, such as an increased accumulation of lipofuscin)³⁴; such changes also could account for some of the hyperAF changes observed in these areas of nGA. In the context of intermediate AMD, hyperAF have been found most often to colocalize with hyperpigmented clumps overlying large drusen, but can also occur in areas without drusen or hyperpigmentary changes.^{10,19,20,22} As observed in our previous study, hyperpigmentary changes were often present in these areas of nGA²⁵ and, thus, may account for some of the hyperAF changes observed. The hypoAF changes in an area of nGA graded as having mixed AF, though not appearing as a sharply delineated area of hypoAF, are likely to represent the same pathological changes of RPE loss as areas of drusen-associated atrophy. This is also not surprising as areas of nGA often show traces of increased signal transmission below BM, which in areas of GA correspond closely with the area of RPE loss.^{29–31}

It also is possible that the hyperAF changes in these areas of nGA represent areas of outer retinal disruption, since loss of retinal photopigments with outer retinal disruption can create a window defect that allows the fluorescent signals from the underlying RPE to be better visualized.¹¹ This would be consistent with our previous study that showed that ISe band disruption without a complete loss of the RPE often was present in areas of nGA.²⁵ Given the close association between the degree of ISe band disruption and microperimetric sensitivity,^{35,36} this also would be consistent with our recent study that observed that areas of nGA were characterized by poorer microperimetric sensitivity than nonatrophic areas.²⁶ However, future studies are needed to examine this through photopigment bleaching during FAF imaging.¹¹

These findings provided further understanding into the pathological changes in areas of nGA, areas that have been found subsequently to develop drusen-associated atrophy.²⁵ These changes provide an ability to better characterize the disease severity in the early stages of AMD. In this study, we also observed that areas of nGA that were graded as exhibiting mixed AF developed drusen-associated atrophy approximately 5 months earlier than areas graded as being hyperAF. Therefore, the FAF characteristics of the nGA area could also potentially assist in determining the time to the development of drusen-associated atrophy.

There are several limitations that need to be acknowledged when considering the findings of this study. Firstly, the

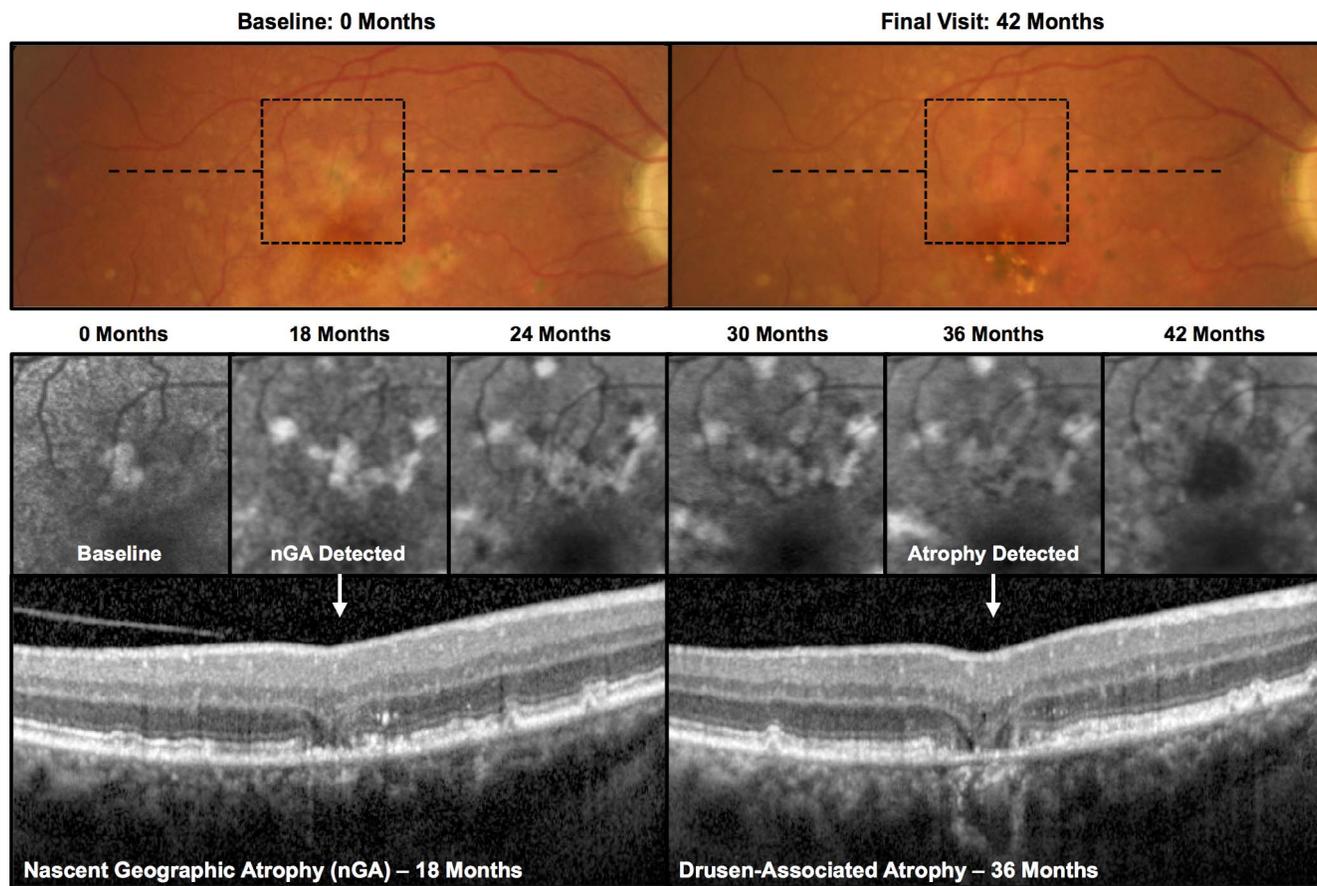


FIGURE 3. Example of the longitudinal changes in FAF characteristics preceding the development of atrophy. Top row shows CFPs for the baseline (0 months) and final visit (42 months), where an area of GA is only visible by the final visit (indicated by the *black dashed box* at 42 months). Middle row shows FAF images that are magnified from the areas outlined by the *black dashed boxes* in the CFPs at various time intervals, where the corresponding SD-OCT scans are shown (*bottom row*; the location of these scans are also outlined on the CFPs as *dashed black lines*) for the development of nGA at 18 months and drusen-associated atrophy detected on SD-OCT at 36 months. The FAF imaging revealed that the area of nGA (18 months) was characterized by predominantly hyperautofluorescent changes, while the area of drusen-associated atrophy (36 months) was characterized by the presence of hyper- and hypoautofluorescent changes, which also is unlike the area of GA (42 months) that appears as a clearly demarcated area of hypoautofluorescence.

different SD-OCT volume scan protocol used at the two different sites in the longitudinal study may have influenced the rate of detecting nGA, although the purpose of this study was to describe the FAF characteristics in areas of nGA that have been detected, not the prevalence or incidence of nGA. Interestingly, the percentage of nGA was not dissimilar in the two cohorts (data not shown), and thus, we do not think that the different scanning protocols influenced our conclusions. Secondly, the limited sample size of the longitudinal study does not permit a conclusive evaluation of the changes in FAF characteristics in areas developing drusen-associated atrophy. We were only able to report what we observed in the eyes in this study, and used them predominantly for the purpose of providing categories to grade the FAF characteristics in areas of nGA in the cross-sectional study. The limited sample size in the longitudinal study was attributed mainly to the exclusion of

eyes that did not have FAF images of sufficient quality at every visit between the first detection of nGA to the first detection of drusen-associated atrophy detected on SD-OCT (approximately 5 visits on average), although we observed a similar pattern of changes in FAF characteristics in these eyes as described in this study (not reported). The finding of insufficient FAF image quality in the longitudinal study also prompted a more careful acquisition of FAF images in the later cross-sectional study. Specifically, we avoided the use of applanation tonometry and used ocular lubricants before obtaining FAF images to minimize the influence of ocular surface changes on the image quality. Finally, the qualitative and subjective nature of grading the FAF characteristics by a single observer may limit the accuracy of determining these changes. In spite of this, the difference in the distribution of the FAF characteristics between the two types of atrophic areas were quite marked, but future studies are required to enlist quantitative methods of comparing the level of autofluorescence in these atrophic areas to examine this further. Future studies also are required to compare these quantitative changes on FAF with changes on CFP using precise image registration techniques to understand the pathological changes occurring in these atrophic areas.

In summary, areas of nGA were characterized most commonly by the presence of hyperAF and hypoAF changes, while areas of drusen-associated atrophy most often appeared

TABLE 2. The FAF Imaging Characteristics in Areas of nGa and Drusen-Associated Atrophy Detected on SD-OCT

	HyperAF	Mixed AF	HypoAF
Nascent geographic atrophy	4 (8%)	31 (63%)	14 (29%)
Drusen-associated atrophy detected on SD-OCT	-	2 (14%)	12 (86%)

hypoAF on FAF imaging. These findings provided further understanding into the FAF characteristics of areas undergoing atrophic changes and assisted in the characterization of disease severity in the early stages of AMD.

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