Longitudinal Associations Between Microstructural Changes and Microperimetry in the Early Stages of Age-Related Macular Degeneration

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PURPOSE. To determine whether longitudinal changes in mesopic visual function on microperimetry occurred independent of its associations with microstructural parameters on spectral-domain optical coherence tomography (SD-OCT) in the early stages of AMD.

METHODS. Forty-one AMD eyes underwent microperimetry testing and SD-OCT scans over a 12-month period at 6-month intervals. Microstructural parameters analyzed included the retinal pigment epithelium-drusen complex (RPEDC) layer thickness, number of hyperreflective foci (HF) and their inner retinal migration (represented by a weighted axial distribution score; AxD), and the number of atrophic areas.

RESULTS. Microperimetric sensitivity was 0.29 dB (95% confidence interval [CI] = −0.38 to −0.20 dB, \( P < 0.001 \)) and 0.13 dB (95% CI = −0.22 to −0.03 dB, \( P = 0.008 \)) lower in each sector for every 10-µm higher RPEDC layer thickness and 1-HF present, but was not associated with the AxD score or the number of atrophic areas present (\( P \leq 0.464 \)). However, each 10-µm greater RPEDC layer thickness and 1-HF present was not independently associated with a further decline in sensitivity (−0.08 dB/year, 95% CI = −0.24 to 0.07 dB/year, \( P = 0.288 \) and 0.09 dB/year, 95% CI = −0.06 to 0.24 dB/year, \( P = 0.242 \), respectively) over time when accounting for the association between RPEDC layer thickness and number of HF with microperimetric sensitivity.

CONCLUSIONS. Longitudinal changes in mesopic visual function measured on microperimetry paralleled changes in the microstructural changes over a 12-month time frame, without any changes occurring independent of the associations between structure and function alone.

Keywords: age-related macular degeneration, microperimetry, optical coherence tomography, longitudinal

Novel interventions are now being developed to target AMD in its early stages,¹–⁴ as it is recognized that an effective treatment for preventing the development of advanced complications and/or vision loss would have a significant public health impact.⁵ However, a major challenge when evaluating such new interventions for the early stages of AMD is the lack of clinical biomarkers that can act as surrogate endpoints for the clinical endpoint of vision loss and/or development of advanced complications in AMD. This means that current clinical studies require large sample sizes and long durations of follow-up to determine treatment efficacy, and better clinical biomarkers of the early stages of AMD are clearly required.

Although various pathologic features and parameters visualized or quantified on imaging modalities such as spectral-domain optical coherence tomography (SD-OCT) have recently been reported to be associated with the development of advanced AMD,⁶–⁹ previous studies have also observed that visual function changes precede the development of neovascular¹⁰–¹³ or atrophic complications.¹⁴–¹⁶ However, none of these measures have been accepted as a surrogate endpoint by regulatory bodies such as the US Food and Drug Administration. Among these visual function measures, we have previously investigated the measure of visual sensitivity to luminance increments using automated perimetry and observed that it decreased at a significantly higher rate preceding the development of geographic atrophy (GA), thus highlighting the potential utility of this measure for providing a topographical evaluation of eyes with the early stages of AMD.¹⁴ Using fundus-tracing during these perimetric examinations with a technique commonly referred to as microperimetry, we recently found that subtle functional decline over a 12-month period was detected in eyes that appeared either stable or had progressed within the early stages of AMD based on changes in the extent of drusen and/or pigmentary abnormalities on color fundus photographs (CFP); however, the long-term implications of this decline remained to be determined.¹⁵

It is also recognized that subtle changes in microstructural features occur in the early stages of AMD, despite not easily appreciated on clinical examination or on CFPs.¹⁶,¹⁷ Such changes can be visualized and quantified on high-resolution imaging modalities such as SD-OCT, including changes to the extent and morphology of drusen,¹⁸–¹⁹ pigmentary abnor-
malities, atrophic changes (including nascent GA [nGA] and drusen-associated atrophy) and the integrity of the photoreceptor inner-segment ellipsoid (ISe) band. Previous studies have also observed that changes in some of these microstructural features are strongly associated with changes in mesopic microperimetric sensitivity, although it is not possible to conclude whether any of these parameters represent the underlying pathologic changes that are contributing to the decreased visual sensitivity.

However, it remains to be determined whether changes in visual function on microperimetry simply parallel changes in microstructural changes occurring in corresponding locations over time, or whether some degree of functional change occurs independent of the structural changes. In the latter case, microperimetric sensitivity could then provide unique insights into the disease state and be used as an independent marker of disease severity and risk of progression. Conversely, parallel changes in structure and visual function would suggest that the structural parameters could provide an indication of the changes in microperimetric sensitivity expected to occur.

This study, therefore, examined the associations between visual function changes on mesopic microperimetry and longitudinal changes in SD-OCT microstructural parameters, including drusen volume, pigmentary changes (visualized on SD-OCT as hyperreflective foci [HF] and its inner retinal migration and proliferation, and atrophic changes (including nGA and drusen-associated atrophy detected on SD-OCT) in participants with the early stages of AMD. All these parameters have been observed to be significantly associated with the future development of advanced AMD and may therefore be independently associated with the longitudinal rate of change in microperimetric sensitivity.

METHODS

This study was approved by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital and conducted in adherence with the Declaration of Helsinki. Written informed consent was obtained from all participants after providing an explanation of all the test procedures.

Participants

The inclusion criteria for all participants in this study included being 50 years of age or older, and having a best-corrected visual acuity of 20/40 (or 0.50 logMAR) or better, a refractive error of no greater than ±6.00 diopter (spherical equivalent), and drusen ≥125 μm (with or without pigmentary changes and reticular pseudodrusen) in the study eye. When both eyes met the inclusion criteria, the eye with the better visual acuity was chosen as the study eye. The exclusion criteria for any study eye include the presence of GA or choroidal neovascularization (CNV) on CFP, glaucoma, any corneal pathology that could compromise vision, diabetes, uncontrolled hypertension, amblyopia, neurologic or systemic disease affecting vision, or any medication known to affect retinal function. Participants were also excluded if they had any physical and/or mental impairment preventing them from participating in this study or an inability to sign a consent form. All AMD participants were seen at three visits over a 12-month period, at 6-monthly intervals. This cohort contributed to previously reported data.

Procedures

In this study, all participants first performed visual acuity measurements, then microperimetric examinations, followed by multimodal imaging and then clinical examination by a senior retinal specialist (RHG) during each visit.

Microperimetric Examination

All participants underwent mesopic microperimetry examinations using the Macular Integrity Assessment (MAIA; Center-Vue, Padova, Italy) microperimeter using a procedure that has been outlined in detail previously, where participants were not preadapted to either the background luminance of the room or the microperimeter, and the room illumination was switched off just before each examination commenced to ensure the same degree of light exposure. In brief, pupillary dilation using one drop of 1% tropicamide and one drop of 2.5% phenylephrine to achieve a pupil diameter of at least 6 mm was performed before the examinations. Identical verbal instructions were then provided to all participants regarding how to perform the microperimetry examination.

A line-scanning laser ophthalmoscope (SLO) with a superluminescent diode illumination with a central wavelength of 850 nm was used to achieve fundus tracking on the MAIA microperimeter. The entire fundus image was used as a reference when performing fundus tracking, with fundus images captured at 25 frames per second. A red circular fixation target of 1° diameter was used in this study, and Goldman III stimuli were presented for 200 ms against a background of 1.27 cd/m² using a 4 to 2 threshold strategy. The maximum stimulus luminance was 318 cd/m², creating a dynamic range of 36 dB. A customized stimulus grid (Centre for Eye Research Australia AMD 6º grid) consisting of 37 points located at 0º, 1º, 2.5º, 4º, and 6º from fixation was used in this study, designed by our group specifically for the assessment of the macular region in AMD.

In this study, two consecutive examinations during each visit were performed on the study eye for all participants with approximately 3 minutes of rest given between each test to minimize the effect of fatigue. Test reliability for the second examination was assessed by the frequency of false-positive responses, measured by presentations of suprathreshold stimuli to the physiological blind spot, which was manually located on the microperimeter before the presentation of the first stimuli. Any tests with false-positive responses of more than 25% were considered unreliable and were repeated. This cutoff was chosen because the microperimeter presents a false-positive stimulus approximately every 1 minute, and there are typically only four to five false-positive stimuli presented in each test given the short duration of the examinations (approximately 5.5 minutes on average).

Imaging and Image Analysis

Multimodal imaging was performed at all visits and included CFP, near-infrared reflectance (NIR) and fundus autofluorescence imaging, and SD-OCT volume scans using a Spectralis HRA+OCT device (Heidelberg Engineering, Heidelberg, Germany). The volume scans were performed over a 20 × 20º area centered on the fovea, with 49 equally spaced horizontal B-scans used. Each B-scan contained 1024 A-scans and was set to average 25 frames each. The automatic follow-up scan placement function of the Spectralis HRA+OCT device was used to obtain scans at the same location as the first volume scan on all follow-up visits. The unprocessed volume scans were deidentified, and then transferred to the Duke University for image analysis.

Layer segmentation for each B-scan was then performed semiautomatically using definitions and markings outlined in our previous publication, and carefully reviewed and corrected manually by a grader if required. Segmentation of the layers also included one additional segmentation line used to delineate the inner aspect of the external limiting membrane.
Hyperreflective foci were semiautomatically identified in a two-step process. In the first step, we used a set of eight matched filters of different sizes and shapes, which corresponded to different manifestations of HF on OCT images. Potential HF locations in each B-scan were associated with corresponding positions with highest filter response in the matched filter paradigm. The potential HF were then automatically narrowed down using a set of rules emulating visual cues used by graders in marking HF: (1) the grayscale intensity of the HF ≥75% of the intensity of RPEDC in the nearby lateral region, (2) the size of a lesion is ≥180 μm², and (3) the HF is located below the IPL-INL boundary and above the RPEDC. After automatic segmentation, both the HF and layer segmentations were manually corrected by an expert grader in DOCTRAP software.

To quantify inner retinal shift or migration of HF, a novel parameter, the axial distribution (AxD) score, was calculated for each eye at each study visit. The AxD score, an adaptation and quantitative extension of a previous qualitative metric, is a weighted score defined as the sum of all HF detected in each sector of an eye at each visit, with each single HF multiplied by a correction factor (γ) whose value was determined by its location: adjacent to the RPEDC or within the EZ+OS (γ = 1), MZ (γ = 2), ONL (γ = 3), OPL (γ = 4), and INL (γ = 5). This is a minor change from the original metric, in which HF with its center located within the RPEDC were considered (γ = 1), but were not considered to be HF in this study due to the difficulty of distinguishing them from other changes occurring at the RPE. Instead, this study considered HF located within the EZ+OS to be (γ = 1). The original metric also considered the RNFL, RGC+IPL, and INL combined as (γ = 5), instead of the INL only in this study, because no HF were observed in the other layers in our previous study and we sought to avoid the improper automatic identification of blood vessels as HF; all other designations for the calculation agreed. In cases in which layers are missing due to pathologic changes, the location of a HF is defined as first visible layer above the HF. The final AxD value is the total sum of the products divided by the total number of detected HF, which weighs this score against the confounding effect of different quantities of HF between visits.

The number of atrophic areas, including nGA or drusen-associated atrophy detected on SD-OCT, was also identified in the volume scans. Briefly, nGA was defined as the presence of features including the subsidence of the OPL and INL, and/or the development of a hyporeflective wedge-shaped band within the limits of the OPL, and was defined as an area with a loss of the RPE and inner nuclear layer (ONL) and development of a hyporeflective wedge-shaped band within the limits of the OPL. Drusen-associated atrophy detected on SD-OCT was defined as an area with a loss of the RPE and INL bands, resulting in increased signal transmission below Bruch's membrane (BM) that is accompanied by loss of the ELM and outer nuclear layer (ONL) in this area. Note that drusen-associated atrophy was defined using SD-OCT, and GA (an exclusion criterion for this study) was defined on CFP.

Data and Statistical Analysis

The average microperimetric sensitivity and RPEDC layer thickness, AxD score, and number of HF and atrophic areas were then determined in five sectors using the inner two rings of a modified Early Treatment for Diabetic Retinopathy grading template, as outlined in Figure 2. These parameters were

**Figure 1.** Automated segmentation of OCT scans (unmarked, left) to obtain individual layer thicknesses and automated identification of HF (orange asterisks). The segmented layers are RNFL, GCL+IPL, INL, OPL, ONL, MZ, EZ+OS, and RPEDC.

**Figure 2.** Determination of sectors used to average microperimetric sensitivity and microstructural parameters from the OCT scans, shown on an example of an eye included in this study with drusen and pigmentary changes (where the colored numbers represent the measured sensitivity in decibels overlaid onto a near-infrared reflectance image obtained by the microperimeter); the inner and outer circles are 1.6 and 5.6 mm in diameter, respectively.
defined within a sector as opposed to being defined at the exact locations sampled by a stimulus so as to investigate whether pathologic changes had a more generalized impact on visual function. The inner and outer circles were 1.6 and 3.6 mm in diameter, respectively, so as to allow each sector to cover equal areas of retina. These sectors were also chosen to allow corresponding areas of structure and function to be compared.

Random coefficient models were used to determine whether changes in microperimetric sensitivity over time paralleled changes in the microstructural parameters, or whether there was some degree of functional change that occurred independent of the structural changes. Briefly, these models are used to account for the hierarchical (e.g., individual sectors nested within an eye), repeated-measures nature of the data. They incorporate both random intercepts (representing different baseline values) and random slopes (representing different rates of change) in a single model, and accommodate for the correlation of the residuals that are expected to occur with repeated measures, $^{56,57}$ and are thus an ideal statistical model for evaluating these data.

First, a model was built to examine the association between the microstructural parameters (HF, AxD, RPEDC, and atrophic areas) and microperimetric sensitivity and included data from all three visits (Model 1). The microstructural parameters and time (indicating the visit) were included as fixed effects. The intercept and slope for each patient were included as a random intercept, time, and each of the microstructural parameters (HF, AxD, RPEDC, and Atrophic Areas), respectively.

\[
\text{Sensitivity}_{abc} = \beta_0 + \beta_1 \times \text{Time}_{abc} + \beta_2 \times \text{HF}_{abc} + \beta_3 \times \text{AxD}_{abc} + \beta_4 \times \text{RPEDC}_{abc} + \beta_5 \times \text{Atrophic Areas}_{abc} + \gamma_{0c} + \gamma_{1c} \times \text{Time}_{abc} + \gamma_{0b/c} + \gamma_{1b/c} \times \text{Time}_{abc} + \epsilon_{abc}
\]

Where $\beta_0$ through $\beta_5$ represent the fixed effects associated with the intercept, time, and each of the microstructural parameters (HF, AxD, RPEDC, and Atrophic Areas), respectively. $\gamma_0$ and $\gamma_1$ represent the random patient effects associated with the intercept and time respectively. $\gamma_{0b/c}$ and $\gamma_{1b/c}$ represent the random sector nested within patient effects associated with the intercept and time respectively, and $\epsilon_{abc}$ represents the residual.

Another model was then built to examine whether the extent of the microstructural parameters at each time point were also independently associated with the longitudinal rate of change in microperimetric sensitivity, independent of the associations between the microstructural parameters and microperimetric sensitivity alone (Model 2). This was achieved by adding two-way interactions between the microstructural parameters found to be significantly associated with microperimetric sensitivity in Model 1 and time as fixed effects; the same random effects as the previous model were included. The general form of the model was similar to Model 1, but including the two-way interactions described (not shown).

### Results

Forty-one consecutive AMD participants were originally included in this prospective study. One participant developed CNV in the study eye during the follow-up period, and another participant did not have SD-OCT images of sufficient quality at all visits, and therefore both were excluded from subsequent analyses. No participants in this study developed GA. The remaining 39 participants included in the analysis were on average 69.6 ± 9.5 years of age (range, 50–88), were female for 26 participants (67%), and were seen at 6.6 ± 1.0 months and 12.4 ± 0.8 months from baseline at the second and third visits, respectively. The clinical characteristics of these participants are outlined in Table 1. Of interest, 12 and 8 of the 39 eyes exhibited a change of less than –1 dB and more than 1 dB, respectively, in mean microperimetric sensitivity between the first and third visits.

### Parameters Associated With Microperimetric Sensitivity Over Follow-up (Model 1)

A random coefficients model was developed to investigate which parameters were associated with the microperimetric sensitivity of each sector over the three visits, and the results are shown in Table 2. This model showed that microperimetric sensitivity was significantly associated with the number of HF ($P = 0.008$) and the RPEDC layer thickness ($P < 0.001$), but not the AxD score ($P = 0.511$) in each sector. These significant associations indicate that every increase in 1 HF or 10-μm increase in RPEDC layer thickness were independently associated with a 0.15-dB and 0.29-dB decrease in microperimetric sensitivity in a sector, respectively. Microperimetric sensitivity was negatively, but not significantly, associated with the number of atrophic areas (nGa or drusen-associated atrophy detected on SD-OCT) ($-0.18$ dB, 95% confidence interval [CI] = –0.65 to 0.39, $P = 0.464$). There were 11, 15, 11, and 11 atrophic areas in each of the four visits.
and 14 atrophic areas at the baseline, 6-month, and 12-month visits, respectively, where 23 (61%) of these 38 atrophic areas in total were not directly sampled by any micropertimetric stimulus. Micropertimetric sensitivity was still not significantly associated with the number of atrophic areas when we included only areas sampled by micropyrimetry (−0.57 dB, 95% CI = −1.19 to 0.46 dB, $P = 0.384$; full model not shown). Micropertimetric sensitivity was also not significantly associated with time in this model ($P = 0.669$), suggesting that changes in sensitivity in these eyes with the early stages of AMD did not occur over time independently of structural changes (also meaning that changes in micropertimetric sensitivity did not precede structural changes).

### Parameters Associated With the Rate of Change in Micropertimetric Sensitivity (Model 2)

Another random coefficients model was then built to investigate whether the extent of the microstructural parameters at each time point were independently associated with the longitudinal rate of change in micropertimetric sensitivity, independent of the significant associations between the microstructural parameters and micropertimetric sensitivity alone. Only the number of HF and the RPEDC layer thickness were included as fixed effects in this model because only these parameters were found to be significantly associated with micropertimetric sensitivity in Model 1. The results are presented in Table 3, and show that neither microstructural parameter exhibited a significant interaction with time ($P \geq 0.242$), in addition to their associations with micropertimetric sensitivity alone ($P \leq 0.004$). These results indicate that changes in these microstructural parameters were not independently associated with changes in micropertimetric sensitivity beyond what could be expected by the associations between them. For example, if the estimate of “RPEDC × Time” parameter was −0.10 dB, a 10-μm increase in RPEDC thickness over 12 months would be expected to be associated with a −0.24-dB decrease in micropertimetric sensitivity from the estimate of the “RPEDC” (Table 3), with a further decrease of −0.10 dB from the “RPEDC × Time,” and thus an estimated decrease of −0.34 dB would be expected. Had the estimate of “RPEDC × Time” parameter been −0.10 dB, we would have also expected that a 0-μm change in RPEDC thickness over 12 months would be associated with no change in micropertimetric sensitivity from the estimate of the “RPEDC” (Table 3), but a decrease of −0.10 dB from the “RPEDC × Time.” However, the absence of a significant interaction between “RPEDC” and “Time” ($P = 0.288$; Table 3) indicates that the estimated change in micropertimetric sensitivity is associated with the “RPEDC” parameter alone. Thus, these results collectively indicate that changes in micropertimetric sensitivity appeared to parallel changes in the RPEDC layer thickness and number of HF present.

### Examples of Findings

Two examples from this study are shown to illustrate the parallel between the longitudinal changes in micropertimetric sensitivity and changes in the number of HF and RPEDC thickness (Fig. 3). In the first example, a 2.5-dB decrease in micropertimetric sensitivity was associated with a 37-μm increase in average RPEDC thickness and increase in 6 HF in the central sector (Fig. 3A). In the second example, a 4.5-dB improvement in micropertimetric sensitivity was associated with a 45-μm decrease in average RPEDC thickness and decrease in 13 HF in the central sector (Fig. 3B).

### Discussion

In this study, we observed that longitudinal changes in mesopic micropertimetric sensitivity in eyes with the early stages of AMD paralleled changes in microstructural parameters of the RPEDC layer thickness and number of HF, but not AxD score or the presence of atrophic changes. These findings are consistent with previous cross-sectional studies examining mesopic micropertimetric sensitivity and drusen load23,25,38 and HF.25–27 However, the average micropertimetric sensitivity in a sector was not significantly associated with the number of atrophic areas, as defined on SD-OCT in this study. Although this may appear inconsistent from our recent study that found reduced micropertimetric sensitivity overlying atrophic areas,23 these findings are not incompatible. Specifically, this study did not seek to examine only eyes in which an atrophic area was sampled by a micropertimetric stimulus, unlike our previous study,24 but examined the averaged micropertimetric sensitivity over sectors that were 2.0 mm² in area. Such averaging in addition to the small number of atrophic areas present (and even smaller numbers sampled by a micropertimetric stimulus) does not provide sufficient statistical power or permit a systematic evaluation of whether micropertimetric sensitivity is reduced in atrophic areas (nor was it the primary aim of this study, but was the primary aim in our previous study24), but potentially highlight the localized nature of functional changes in these atrophic areas.

In this study, we had hypothesized that it would be possible for some degree of changes in micropertimetric sensitivity to occur beyond the associations with microstructural changes alone, based on observations that changes in micropertimetric sensitivity occurred longitudinally despite no changes in the pathologic features assessed on CFP (and that no significant changes in micropertimetric sensitivity occurred in a group of healthy participants over this period as well).15 and that functional changes had been observed to precede the development of advanced AMD.10–11 However, we did not observe longitudinal changes in mesopic micropertimetric sensitivity that occurred independent of changes associated with the microstructural parameters alone, including the increase or decrease in the extent of drusen18,19 and pigmentary abnormalities8 that have been observed to occur without the development of advanced AMD. This is also not completely unexpected due to the close association between mesopic micropertimetric sensitivity and microstructural changes observed in cross-sectional studies previously.25–27 Thus, the findings of this study suggest that RPEDC layer thickness and the number of HF could be used as biomarkers of micropertimetric sensitivity that can be rapidly and objectively acquired with imaging devices that are ubiquitous in clinical settings, unlike the assessment of micropertimetric sensitivity that is both subjective and requires additional equipment. However, future studies are required to verify this in an independent cohort. In addition, measurements of
FIGURE 3. Examples of changes in microperimetric sensitivity with changes in RPEDC thickness and number of HF. (A) In the first example, a 37-μm increase in average RPEDC thickness and increase in 6 HF in the central sector were associated with a −2.5-dB decrease in microperimetric sensitivity. B-scans on SD-OCT are shown through an area with the highest RPEDC elevation at the first and third visits, illustrating an increase in the drusen-associated RPE elevation over time. (B) In the second example, a 45-μm decrease in average RPEDC thickness and decrease in 13 HF in the central sector were associated with a 4.5-dB increase in microperimetric sensitivity. No atrophic changes were observed to have developed on SD-OCT, and B-scans are shown through an area that subsequently exhibited regression of drusen between the first and third visits. These examples illustrate the observed correspondence of functional change with structural changes.
microperimetric sensitivity can be affected by other conditions, such as glaucoma or other neurologic conditions, and can only sample localized areas (sampling approximately 1% of the total retinal area within the central 3.6 mm in this study), whereas the microstructural parameters on SD-OCT imaging can provide a more specific indication of the pathologic changes that may be occurring, although such parameters should not be taken to represent biology. Specifically, the parameters in this study, including drusen volume,\textsuperscript{3,9} HF proliferation and inner retinal migration,\textsuperscript{6} and nGA,\textsuperscript{7} have each been reported to be associated with the development of advanced complications of AMD. However, the findings of this study do not preclude the potential of other visual function measures as biomarkers for the future development of advanced AMD (such as dark adaptation, shown recently to be associated with incident early AMD\textsuperscript{9,9}), but that future studies should continue to develop and evaluate potential visual function measures that can fulfill this purpose. This study also does not address whether either microperimetric sensitivity or the structural parameters can act as specific outcome markers to evaluate interventions that may seek to target different endpoints, but merely sought to investigate the potential for microperimetric sensitivity to be a surrogate endpoint for the development of advanced AMD by evaluating its longitudinal associations with the structural parameters included in this study.

It should be noted that these findings do not exclude the utility of microperimetry in the evaluation of disease progression in the early stages of AMD, because a caveat of this study is the limited follow-up duration. This means that the potential of microperimetric sensitivity as an independent predictor for the risk of advanced AMD development could not be determined, and future studies are required to examine this further. However, microperimetry could still be useful for the targeted assessment of longitudinal changes in visual sensitivity within areas such as nGA, where functional changes may not be simply associated with the RPEDC layer thickness and number of HF present alone. We did not include the integrity of the ISe band on the SD-OCT and reticular pseudodrusen (RPD) as parameters examined in this study due to the current limitations in quantifying these parameters using automatic image analysis techniques, and also did not include photoreceptor outer segment (OS) length due to its close correlation with the RPEDC thickness, and we did not find a significant association between the OS length and microperimetric in this study (data not shown) and our previous study.\textsuperscript{25}

An inherent limitation in this study is the reliance on hard thresholds when defining HF, which were determined in consultation with a clinical investigator of this study to improve on the qualitative descriptions used in previous publications.\textsuperscript{6} For example, although we set the minimum HF size as 180 \(\mu\text{m}^2\), we acknowledge that a hyperreflective lesion \(\leq 179 \mu\text{m}^2\) in size could be an HF as well. Further studies are required to determine whether soft thresholds (e.g., fuzzy logic) can improve the identification of HF over the hard thresholds used in this study. We also encountered cases in this study in which the correct identification of retinal features and/or layer boundaries for segmentation were uncertain, such as distinguishing whether a lesion was an HF or a continuation of a druse (Fig. 4). However, these cases were all reviewed and discussed by the investigators of this study, before a collective judgment was achieved. The ability to quantify all the hyperpigmentary changes present in an eye was also limited by the nature of SD-OCT imaging and the image-processing techniques used to detect them, where HF were not considered to be present unless they exhibited an increased reflectivity relative to the nearby RPEDC and were located above the RPEDC segmented line. Hyperpigmentary changes that may have been present as less reflective lesions and/or lesions at or below the RPEDC segmented line would therefore have been missed. However, this criterion was required to optimize the sensitivity and specificity of detecting HF on SD-OCT, and therefore future studies may benefit from using multimodal imaging when seeking to quantify the full extent of hyperpigmentary changes present. In addition, HF may also not have been detected if it was located between the B-scans of the SD-OCT volume scan protocol used in this study, which were spaced approximately 120 \(\mu\text{m}\) apart. Minor registration errors that may have occurred with the automatic image alignment feature could also have contributed to errors in detecting HF over time. It also should be recognized that variations in retinal magnification or axial length between individuals were not accounted for when measuring the RPEDC thickness and should thus be considered as a limitation, but the evaluation of its longitudinal changes within the same eye of an individual in this study should have minimized its influence.

Another limitation in this study was that the lens status was not recorded or quantified, which may have resulted in a generalized decrease in microperimetric sensitivity over time. However, the absence of a significant association between sensitivity and time after accounting for changes in the microstructural parameters suggests that the lens status was unlikely to have had an independent and statistically significant impact on the changes in microperimetric sensitivity over this period. Finally, other limitations when interpreting the findings of this study are the limited sample size and follow-up duration, although the strong associations observed in this study provide...
References


