Impact of Reticular Pseudodrusen on Microperimetry and Multifocal Electroretinography in Intermediate Age-Related Macular Degeneration

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PURPOSE. To examine the influence of reticular pseudodrusen (RPD) on retinal and visual function in intermediate AMD using multifocal electroretinography (mFERG) and microperimetry.

METHODS. In a prospective cross-sectional study, microperimetry and mFERG testing, followed by color fundus photography, near-infrared reflectance imaging and spectral-domain optical coherence tomography (SD-OCT) scans were performed in 120 eyes from 60 participants with bilateral intermediate AMD. The number of subfields with pigmentary changes and RPD within the central 3-mm diameter of the Early Treatment of Diabetic Retinopathy Study (ETDRS) grid and drusen cube root volume within the central 3-mm diameter was determined. The influence of these pathological features on microperimetry and mFERG in this region were examined.

RESULTS. Microperimetric sensitivity was not significantly associated with the presence and extent of RPD ($P = 0.068$), but with drusen volume and extent of pigmentary changes ($P < 0.001$ for both). However, the presence and extent of RPD was independently and significantly associated with mFERG implicit time, along with drusen volume and the extent of pigmentary changes ($P \leq 0.023$). The mFERG response amplitude was not significantly associated with the presence and extent of RPD ($P = 0.130$).

CONCLUSIONS. The presence and extent of RPD was associated with functional changes on mFERG implicit time, but not mFERG response amplitude or microperimetry. These findings suggest that the presence of RPD in eyes with intermediate AMD has a significant influence on cone-mediated neuroretinal function, without a significant influence on mesopic visual function as determined on microperimetry.

Keywords: AMD, spectral-domain optical coherence tomography, microperimetry, multifocal electroretinography

The presence of drusen is the hallmark feature of AMD, where drusen characteristics such as size or extent, along with the presence of pigmentary abnormalities have traditionally been used to determine the risk of progression to the advanced stages of AMD.1–3 More recently, the feature of reticular pseudodrusen (RPD) has also been increasingly recognized as another important risk factor for the development of advanced AMD.4–9

On spectral-domain optical coherence tomography (SD-OCT), RPD are visualized as hyperreflective signals located above the retinal pigment epithelium (RPE) band, which can also be accompanied by disruption of the inner segment ellipsoid (ISe) band.10–13 Some studies using adaptive-optics scanning laser ophthalmoscopy (AO-SLO) have also observed changes to the reflectivity and density of the cone photoreceptors overlying RPD,14–17 leading some to suggest a potential influence on retinal and visual function.14,15

Investigation into the influence of RPD on retinal and visual function has been performed using different measures. One study examined this using multifocal electroretinography (mFERG),18 an objective electrophysiological measure of retinal function, and reported that there was no significant difference in cone-mediated retinal function between areas with and without RPD in an eye. Other studies have investigated the influence of RPD using microperimetry, a psychophysical measure of visual function, and reported a significant reduction in sensitivity to luminance increment under mesopic conditions for eyes with only RPD (without typical drusen) compared with healthy eyes19 and eyes with only drusen (no RPD).20

To date, these studies have examined eyes where only RPD were exclusively present, yet RPD are often present along with features of the early stages of AMD.6–10,21,22 Therefore, we sought to examine the influence that the presence of RPD had on retinal and visual function in eyes with intermediate AMD using mFERG and microperimetry in this study.

METHODS

This study was approved by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH) and was conducted in adherence with the Declaration of Helsinki. All participants provided written informed consent after an explanation of all test procedures.

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Participants

Participants with AMD were recruited from the medical retina clinic at the Royal Victorian Eye and Ear Hospital (RVEEH; Victoria, Australia) and private ophthalmology clinics and were seen at the Centre for Eye Research Australia as part of a study of the structural and functional changes in the early stages of AMD. All participants were required to be over 50 years of age, have best-corrected visual acuity of better than 20/40 (or 0.30 logMAR) and intermediate AMD in both eyes. Intermediate AMD was defined as an eye having drusen greater than 125 μm with or without pigmentary abnormalities. The exclusion criteria for any participants included the presence of any atrophic changes including geographic atrophy (GA), drusen-associated atrophy detected on SD-OCT or nascent geographic atrophy (nGA). Choroidal neovascularization (CNV), significant cataracts, glaucoma, amblyopia, and any corneal pathology that could compromise vision in either eye. Participants were also excluded if they had diabetes or any neurological or systemic disease affecting vision, if they were taking any medication known to affect retinal or visual function (e.g., hydroxychloroquine), if they had any physical and/or mental impairment preventing them from participating in this study or if they were unable or did not provide written informed consent.

Procedures

The AMD participants underwent measurements of best-corrected visual acuity monocularly after a standardized refraction procedure, using an Early Treatment of Diabetic Retinopathy Study (ETDRS) refraction chart at 4 m. Participants then performed microperimetry and then mfERG recordings. Retinal imaging and clinical examination were performed last to minimize the potential influence of bleached photoreceptors on the functional tests.

Microperimetry Examination

Measurements of sensitivity to luminance increment were obtained using the Macular Integrity Assessment (MAIA; CenterVue, Padova, Italy) microperimeter, in a manner as outlined previously. Firstly, pupillary dilation of at least 6 mm was performed using 1 drop of 1% tropicamide and 1 drop of 2.5% phenylephrine. Identical verbal instructions were then given to all participants regarding how to perform the microperimetry test. The MAIA microperimeter performs fundus tracking at 25 frames per second using the entire fundus as a reference. Visualization of the fundus was achieved using a line-scanning laser ophthalmoscope (SLO), with a super-luminescent diode illumination that has a central wavelength of 850 nm. The fixation target is a red ring of 1° diameter, and Goldman III stimuli were presented for 200 milliseconds against a background of 1.27 cd/m² using a 4-2 staircase threshold strategy. The maximum and minimum luminance of the stimulus was 318 cd/m² and 1.37 cd/m² respectively, creating a dynamic range of 36 dB. A customized stimulus grid (CERA AMD 6° grid) consisting of 37 points located at 0°, 1°, 2.33°, 4°, and 6° from fixation was used in this study, which we designed specifically for the assessment of the macular region in eyes with AMD (Fig. 1).

The frequency of false-positive responses, indicated by the frequency of response to suprathreshold stimuli at the physiological blind spot (manually located on the MAIA before the presentation of the first stimuli) was used to provide an index of test reliability. Any examination with false-positive responses of greater than 25% on any examination was excluded, and were repeated again until the test reliability was less than or equal to 25%. This cut-off was chosen because the MAIA presents a false-positive stimulus approximately every 1 minute, and only four to five false-positive stimuli are typically presented in each test given the short duration of the examinations. In this study, two examinations of the right eye followed by at least one examination of the left eye was performed; the first examination was discarded to avoid the potential influence of a learning effect as previously recommended. All participants were given approximately 3 minutes of rest between each test to minimize the effect of fatigue.

Multifocal Electretinography

The recording protocol for the mfERG was performed as described previously. The Visual Evoked Response Imaging System (VERIS Science 6; ElectroDiagnostic Imaging, Inc., Redwood City, CA, USA) and Dawson-Trick-Litzkow (DTL) thread electrodes were used in this study. Pupillary dilation was already performed prior to microperimetry testing, but
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Table 1. Characteristics of Eyes With and Without RPD in This Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RPD (n = 39)</th>
<th>No RPD (n = 81)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drusen volume, mm</td>
<td>0.28 ± 0.02</td>
<td>0.27 ± 0.03</td>
<td>0.792</td>
</tr>
<tr>
<td>Presence of pigmentary</td>
<td>17 (44%)</td>
<td>35 (45%)</td>
<td>0.975</td>
</tr>
</tbody>
</table>

* Generalized Estimating Equation model analysis between RPD and no RPD.

pupil size was measured again prior to mfERG testing to ensure that it was at least 7 mm in diameter. The mfERG recording protocol involved using 103 retinal-scaled hexagons as the test stimulus (Fig. 1), delivered using a fixation monitoring system (FMS) with a pseudorandom m-sequence (m = 15) set at a rate of 75 Hz. The luminance of the white hexagons was set at 5.33 cd.s.m⁻² and the contrast between the black and white hexagons were approximately 99%; the background luminance was set at 200 cd.m⁻². The fixation target was a cross that was 3° in diameter and 0.6° (20% of the fixation target diameter) in thickness; these parameters were chosen to provide a fixation target that was visible enough while maintaining minimal coverage of the central hexagon. The recorded signal was filtered using a band pass filter between 10 and 100 Hz and was amplified 100,000 times (model 12; Grass NeuroData, Quincy, MA, USA). Any segment contaminated with blinks or eye movements were discarded and rerecorded prior to the completion the entire recording.

Imaging
Color fundus photographs were obtained with a Canon nonmydriatic camera (Canon CR6-45NM; Canon, Saitama, Japan). Spectral-domain optical coherence tomography (SD-OCT) raster scans were performed using the Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) using the 200 × 200 A-scan protocol that covered 6 mm × 6 mm centered on the fovea. Any scan where motion artifacts were present or had an image quality score of less than seven were discarded and immediately repeated to ensure that optimal scans were obtained. Near-infrared reflectance and fundus autofluorescence imaging, and high-resolution line and volume SD-OCT scans were also obtained using a Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) to assist the detection of any signs of advanced AMD; the SD-OCT volume scan was performed using 49 B-scans that covered a 20° × 20° area, using the high-resolution setting and averaging 25 frames for each B-scan.

Analysis of Pathological Features
In this study, pathological features were analyzed and graded only within the central 3-mm diameter in order to allow comparisons with the functional parameters to be made in corresponding regions. Drusen volume were determined using the proprietary algorithm on the Cirrus HD-OCT (version 6.0; Carl Zeiss Meditec), which identified the RPE band and computed a virtual RPE floor, an extrapolation of the RPE geometry in the absence of any local deformation in the band.²⁸ The values for drusen volume within the central 3-mm diameter (or approximately 5.2° radius) were used in this study and a cube root transformation was applied to these values. For pigmentary changes and RPD, the number of subfields of the ETDRS grid within the central 3-mm diameter (total of five subfields) where these features were present was considered as the outcome parameter. Pigmentary changes were defined when both hyperpigmented clumps were visible on color fundus photography as well as hyperreflective foci (HF) visible on SD-OCT scans;²⁹ hypopigmentary changes were not accounted for. The presence of RPD was defined as groups of hyperreflective lesions against a mildly hyperreflective background on near infrared reflectance imaging, with corresponding hyperreflective signals above the RPE band on SD-OCT.¹¹,¹²,³⁰,³¹ At least five or more lesions of definite RPD were required for RPD to be graded as being present in a subfield. In addition, all participants not considered to have any RPD within the central 3-mm diameter were also graded to determine if RPD were present outside this region using the same criteria. One experienced grader performed all the grading in this study of the right and left eye of each participant sequentially, while being masked to the results of the functional measures. Any uncertainty in grading was adjudicated with a senior retinal specialist.

Statistical Analysis
For microperimetry, luminance increment sensitivity of all points within the central 4° (which fell within the central 3-mm diameter) was averaged to provide a mean sensitivity (MS) value in this region. For mfERG, a summed response from the central two rings (corresponding to approximately the central 4° radius, and consists of seven hexagons) was used and N1-P1 response amplitude (density-scaled) and P1 implicit time were used as the outcome parameters; no spatial averaging was applied to the mfERG data.

An independent-samples t-test was used to examine the difference in age between participants with greater than or equal to 1 subfields with RPD in either eye, and participants without any RPD in either eye. Generalized estimating equation (GEE) models were used to examine the difference in structural parameters between eyes with RPD and eyes without RPD, and also the association between the structural parameters and the functional parameters. An exchangeable correlation structure (specifying homogenous correlations between eyes) was used as the type of covariance matrix to account for within-subject correlations because two eyes were used for each participant (because measurements are often correlated between the two eyes), and model-based estimates of the standard errors were used for the parameters. For the analyses of the association between structural and functional parameters, each structural parameter was first entered into the model in a univariate analysis, and parameters found to be statistically significant (P < 0.05) were then entered together into another model in a multivariate analysis. All statistical analyses were performed using commercially available statistical software (IBM SPSS Statistics, software version 21; IBM/SPSS, Inc., Chicago, IL, USA).

Results
A total of 60 AMD participants (70.5 ± 7.2 years, range, 51–84) with intermediate AMD (at least drusen >125 μm) in both eyes (120 eyes) were included in this study, and their characteristics are shown in Table 1. In this study, all participants graded as not having any subfields with RPD within the central 3-mm diameter in this study also did not have RPD outside this region. Among all the participants, 18 (30%) ≥ 1 subfields with RPD in either eye; two (11%) participants had ≥ 1 subfields with RPD in only one eye and 16 (89%) participants had ≥ 1 subfields with RPD in both eyes. Participants with ≥ 1 subfields with RPD in either eye were significantly older than participants who did not have any RPD (76.5 ± 4.3 and 67.9 ± 6.9 years, respectively; P < 0.001). Therefore, all the analyses presented below were also repeated when including partici-
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SD-OCT Parameters Associated With Microperimetry

The univariate analyses showed that age and all SD-OCT parameters, drusen volume, pigmentary changes, and RPD, were significantly associated with microperimetric sensitivity ($P \leq 0.020$). Combining all of these parameters in a multivariate analysis, only age, drusen volume and pigmentary changes were significantly associated with microperimetric sensitivity ($P \leq 0.001$; Table 2), while RPD was no longer significantly associated ($P = 0.068$).

SD-OCT Parameters Associated With Multifocal Electroretinography

For mfERG response amplitudes, the univariate analyses showed that age and RPD exhibited significant associations ($P \geq 0.015$), but drusen volume and pigmentary changes did not ($P \geq 0.056$). Combining those significant parameters in a multivariate analysis, neither parameter remained significantly associated ($P \geq 0.090$; Table 3).

The univariate analyses revealed that all parameters (age, drusen volume, pigmentary changes, and RPD) exhibited a significant association with mfERG implicit time ($P \leq 0.020$). In a multivariate analysis, all parameters still remained significant ($P \leq 0.023$; Table 4). To confirm that the extent of RPD was associated with mfERG implicit time, the multivariate analysis was repeated only for eyes with RPD and the results showed that mfERG implicit time remained significantly associated with the number of subfields where RPD were present ($\beta$ coefficient $= 0.63 \pm 0.20$, $P = 0.002$).

Examples of Findings in This Study

To illustrate the influence of RPD on microperimetry and mfERG parameters, two examples are shown in Figure 2 where both eyes have similar drusen volume in the central 3 mm. In the first example, RPD was present in an eye without any pigmentary changes (Fig. 2B), but mfERG implicit time was greater in the eye with RPD (Fig. 2A). To further illustrate the influence of RPD and pigmentary changes on function, two examples are shown in Figure 3 where both eyes have similar drusen volume in the central 3 mm. In the first example, RPD was present in an eye without any pigmentary changes (Fig. 3A), while the second example was characterized by the absence of RPD but four subfields where pigmentary changes were present (Fig. 3B). Microperimetric sensitivity was worse in the second example with pigmentary changes (Fig. 3B) compared with the first example where it was absent (Fig. 3A), but mfERG implicit time was greater in the first example where RPD was present (Fig. 3A) compared with the second example where it was absent (Fig. 3B).

Discussion

Recent studies investigating the influence of RPD on retinal and visual function have examined eyes where they are present without any typical drusen, allowing a targeted evaluation of this feature. However, RPD are often present in eyes with features of the early stages of AMD, and have been reported to be associated with an increased risk of progression to advanced AMD. Thus, we sought to examine the influence of the presence and extent of RPD, in addition to the features that define intermediate AMD (drusen $>125$ mm, pigmentary abnormalities), on retinal and visual function. In this study, we found that the presence and extent of RPD was associated with changes in mfERG implicit time, but not mfERG response amplitudes or microperimetric sensitivity, after adjusting for age and other pathological features known to influence retinal and visual function.

The association between drusen volume and pigmentary abnormalities with microperimetric sensitivity found in this study is consistent with previous studies in early stages of AMD. However, we did not find a significant association between microperimetric sensitivity and the presence and extent of RPD. A previous study found that eyes with RPD alone had poorer retinal sensitivity than eyes with drusen alone, and thus we had expected the presence and extent of RPD to have a significant influence on microperimetric sensitivity in this study. As our data showed that there was a significant association between pigmentary changes and microperimetric sensitivity in eyes with AMD, it is possible that the presence and extent of pigmentary changes differed between the two groups in the previous study, which may account for the difference in microperimetric sensitivity reported. It is also possible that eyes with RPD alone represent a distinct phenotype with distinct pathological changes (such as outer retinal atrophy) not present in intermediate AMD eyes with RPD, which may account for the reduced microperimetric sensitivity reported.
In this study, we found that mfERG implicit time was associated with the presence and extent of RPD in intermediate AMD. However, a previous study that did not find a significant difference in mfERG responses between areas with and without RPD in an eye, or between the eyes with RPD and healthy eyes,18 and thus we had not expected RPD to have a significant influence on the mfERG responses in this study. Such differing findings have also been observed in mfERG findings of eyes with the early stages of AMD within the literature, where most studies have observed significant functional deficits compared with healthy eyes26,37,38 while some others have not.39,40 It has been suggested these differing findings are likely attributed to the differences in the mfERG recording system and protocol used in those studies. Similarly, the intensity of the white hexagon of the mfERG recording protocol used in our study (5.33 cd.s.m$^{-2}$) was much brighter than that used in the previous study (2.00 cd.s.m$^{-2}$),18 and may thus elicit greater functional deficits and account for these discrepancies.

Although shifts in fixation may have been a limitation when examining the association between the pathological features and functional changes in the central macular region in this study, we believe the techniques employed in this study allowed these comparisons to be valid and the results of this study to be relevant. Firstly, the fundus-tracking feature employed by microperimetry allowed the specified regions within the central 3-mm diameter to be accurately sampled since it compensates for fixation shifts. Secondly, the fixation monitoring system (FMS III) used during mfERG recordings allowed us to discard any segments contaminated by blinks or eye movements. In addition, the portion of the mfERG stimulus pattern analyzed in this study was smaller than the region where the pathological features were analyzed by approximately 1° in radius, and thus small fixation shifts would unlikely have resulted in an area outside the region analyzed to be stimulated.

In an attempt to understand the additional influence of RPD in eyes with intermediate AMD on retinal and visual function, it is important to note that the technique of mfERG and microperimetry measure different aspects of function and we did not find significant correlations between the parameters of these two methods in a recent study.41 Importantly, the mfERG is an electrophysiological measure of suprathreshold responses at photopic levels and the first order kernel responses originate from the cone photoreceptors and bipolar cells,42 while microperimetry is a psychophysical measure of luminance increment sensitivity at mesopic levels, which may be mediated by both rod and cone photoreceptor pathways. Additionally, measurements on microperimetry are not solely influenced by the physiological condition of the retina unlike mfERG, but by the entire visual pathway. Thus, mfERG may be capturing a unique aspect of cone-mediated neuroretinal deficits associated with RPD in eyes with intermediate AMD as observed in this study. Specifically, the association between the presence and extent of RPD with mfERG implicit time, but not response amplitude, suggests an association with neuroretinal functional abnormalities of the cone-mediated pathways rather than cell loss.45 However, our findings are unable to determine the exact mechanisms responsible for these functional changes.

The findings of altered cone photoreceptor appearance overlying RPD visualized on AO-SLO in other recent studies14–17 also supports the association between mfERG implicit time changes and the presence and extent of RPD found in this study. However, these cone photoreceptor changes did not appear to have a significant influence on mesopic visual function, as determined on microperimetry in this study.

A recent histologic study of RPD also suggested that their presence may also be associated with pathophysiological changes of rod photoreceptors, due to the preferential localization of RPD in the perifovea, the area with the highest rod photoreceptor density.44 In this study, we did not examine

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\text{Influence of reticular pseudodrusen (RPD) on microperimetric sensitivity and mfERG implicit time. Two examples are shown where both eyes have similar drusen volume and pigmentary changes are absent in the central 3 mm in both eyes, but RPD is absent in one eye (A) and present in the other (B). The microperimetric sensitivity was similar in both eyes, but mfERG implicit time was greater in the eye with RPD (B) compared with the eye without RPD (A).}
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the influence of RPD on rod-mediated retinal and visual function and future studies are required to examine this, as we have anecdotally observed in our clinical experience that participants with RPD more frequently report symptoms associated with dark adaptation and scotopic dysfunction, which is consistent with a recent report (Flamendorf J, et al. IOVS 2014;55: ARVO E-Abstract 5221). This will not only provide further understanding into the pathological nature of RPD, but its influence on visual function under these conditions.

We also observed that participants with RPD were also significantly older than participants without, and although we have adjusted for age in all the multivariate analyses, we further confirmed the findings of this study by analyzing participants of similar age between the two groups. The findings from these analyses were consistent with those from the whole cohort, and we are thus confident that the difference in age was appropriately accounted for in our analyses.

In conclusion, the presence and extent of RPD in eyes with intermediate AMD was independently associated with worse functional deficits in mfERG implicit time, but not with mfERG response amplitudes and on microperimetry.

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