Reticular Pseudodrusen in Intermediate Age-Related Macular Degeneration: Prevalence, Detection, Clinical, Environmental, and Genetic Associations

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PURPOSE. To determine the prevalence of reticular pseudodrusen (RPD) and their detection using multimodal imaging in patients with bilateral large drusen, and examine their clinical, demographic, environmental, and genetic associations.

METHODS. Three hundred participants with bilateral large drusen (≥125 μm) underwent color fundus photography (CFP), near-infrared reflectance (NIR), fundus autofluorescence (FAF), and spectral-domain optical coherence tomography (SD-OCT) imaging. Demographic information, smoking, and medical history were recorded, and a blood sample was obtained and genotyped to identify the risk alleles of the CFH and ARMS2 genes.

RESULTS. Reticular pseudodrusen were detected in 28.2% eyes of 29.0% participants using NIR and SD-OCT combined, but CFP and FAF detected only 42% and 89%, respectively, of these eyes with RPD. Participants with RPD were significantly older than those without (P < 0.001), but there was no significant difference in sex distribution, smoking history, cardiovascular factors, and minor allele frequency of the CFH gene (P > 0.173). However, the minor allele frequency of the ARMS2 gene was significantly higher in participants with RPD (P = 0.002).

CONCLUSIONS. Reticular pseudodrusen were detected on NIR and SD-OCT in more than a quarter of participants with bilateral large drusen, being often overlooked with CFP. Those with RPD had a higher frequency of the ARMS2 risk variant, and eyes with RPD were more likely to have atrophic changes. These findings are important to consider when managing patients with intermediate AMD.

Keywords: reticular pseudodrusen, subretinal drusenoid deposits, drusen, age-related macular degeneration

The presence of drusen and pigmentary abnormalities are considered to be the hallmark features of AMD, and they confer a risk of developing vision-threatening complications including neovascularization and atrophy. However, it has recently been increasingly recognized that a feature termed reticular pseudodrusen (RPD)1–2 also contributes to this risk.3–5 Specifically, RPD have been reported to occur frequently in eyes with advanced AMD, thereby implicating them as an important risk factor for the development of such complications.6–12 Following its first description in 1990,1 RPD were initially defined as a yellowish and ill-defined interlacing network on clinical examination and/or fundus photography.1,2,5,10,11 In more recent years, imaging modalities including near-infrared reflectance (NIR), fundus autofluorescence (FAF), and spectral-domain optical coherence tomography (SD-OCT) have been used to further characterize these lesions, with SD-OCT imaging localizing these lesions within the subretinal space.8,14 These subretinal drusenoid deposits have also been found to appear as an orderly array of relatively white, dot-like accumulations, rather than exclusively as an ill-defined interlacing network.15 These new imaging modalities have also subsequently showed that RPD are better detected using NIR and SD-OCT than color fundus photography (CFP),7,9,16,17 meaning that their true prevalence may have been underestimated by previous studies that have relied on CFP alone for detection.5,18

No study to date has prospectively evaluated the prevalence of RPD in persons with large drusen (≥125 μm) in both eyes and without advanced AMD (considered to be intermediate AMD19) with NIR and SD-OCT combined. No study has also examined the clinical, environmental, and genetic associations of RPD in intermediate AMD. Characterizing RPD in intermediate AMD is important because it will have important implications for management of these patients, and also potentially for clinical trials seeking to intervene at this earlier stage of disease before vision has become irreversibly lost. This study therefore seeks to determine the prevalence and detection of RPD in persons with intermediate AMD, as well as its clinical, demographic, environmental, and genetic associations.

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**

Participants were recruited from persons referred to the Macular Research Unit at the Centre for Eye Research Australia (CERA) by their eye care providers for consideration of participation in clinical studies being conducted at our research unit. The inclusion criteria for all participants in this study included being 50 years of age or older, having a best-corrected visual acuity of 20/40 (or 0.30 logMAR) or better, and having drusen larger than 125 \( \mu \text{m} \) (with or without pigmentary changes) in both eyes, meeting the definition of intermediate AMD when using a recently proposed clinical classification system.\(^7\) Note that participants with RPD, but without large drusen in both eyes did not meet this inclusion criterion and were not included in this study. The exclusion criteria for any participant included having in either eye geographic atrophy (defined on color fundus photographs as a sharply delineated area of hypopigmentation that is \( >175 \mu \text{m} \) in diameter, in which the choroidal vessels are more visible than in the surrounding areas), choroidal neovascularization, significant cataracts or any corneal pathology that could obscure fundus imaging, diabetes, or uncontrolled hypertension. 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.

**Procedures**

A questionnaire was first administered to obtain demographic information, medical history, and smoking history (never, previous, or current); the medical history included information regarding the diagnosis of hypertension, atherosclerosis, and hypercholesterolemia. Visual acuity measurements were then performed before pupillary dilation, followed by multimodal retinal imaging and clinical examination by a senior retinal specialist. A blood sample was then taken for DNA extraction. Best-corrected visual acuity measurements were performed monocularly using a standardized refraction protocol using an Early Treatment of Diabetic Retinopathy Study refraction chart at 4 m.

**Imaging and Image Analysis**

Multimodal imaging was performed at all visits and included CFP using a nonmydriatic fundus camera (Canon CR6-45NM; Canon, Saitama, Japan). Near-infrared reflectance, FAF, and SD-OCT volume scans were obtained using a Spectralis HRA+OCT device (Heidelberg Engineering, Heidelberg, Germany). Volume scans were performed over the central \( 20 \times 20^\circ \) area, with 49 equally spaced horizontal B-scans used. Each B-scan contained 1024 A-scans and was set to average 25 frames each. In this study, an experienced grader performed the grading of the images from all the participants for all imaging modalities. To minimize bias, the images of all participants for each imaging modality were graded while being masked to other imaging modalities during different sessions. The grading of CFP was performed using OptomizePro (Digital Healthcare Image Management System; Digital Healthcare Ltd., Cambridge, UK) using an established grading system.\(^8\) On CFP, AMD pigmentary abnormalities were defined as the presence of hyperpigmentation and/or hypopigmentation.\(^9\) Reticular pseudodrusen were defined on CFP as being present when pale, ill-defined networks of broad, interlacing ribbons,\(^7\) and/or five or more orderly arrays of relatively white, dot-like accumulations were seen.\(^9\) Reticular pseudodrusen were defined on FAF imaging as a network of five or more round and/or oval lesions that were characterized by decreased FAF signal surrounded by mildly increased FAF signals.\(^7\) On NIR and SD-OCT combined, RPD were considered to be present when five or more hyporeflective lesions were present against a mildly hyperreflective background on NIR imaging that could also be identified as hyperreflective signals above the RPE band on SD-OCT. All participants were required to have good-quality images for all imaging modalities, and were otherwise excluded if images from any imaging modality were deemed to be poor. On SD-OCT, atrophic changes were defined as the presence of nascent geographic atrophy (nGA) and/or drusen-associated atrophy. Briefly, nGA was defined as the presence of features including the subsidence of the outer plexiform layer (OPL) and inner nuclear layer, and/or the development of a hyporeflective wedge-shaped band within the limits of the OPL.\(^9\) Drusen-associated atrophy was defined as an area with a loss of the RPE and inner segment ellipsoids (ISe) bands, resulting in increased signal transmission below Bruch’s membrane and accompanied by loss of the external limiting membrane and outer nuclear layer in this area.\(^9\)

**Genetic Analysis**

Genomic DNA was extracted from peripheral blood leukocytes according to established protocols. For genotyping, 10 ng genomic DNA was amplified using PCR in a multiplex reaction using Hotstart taq polymerase (Bioline, London, UK). A MassEXTEND reaction was undertaken using the designed primers, and samples were spotted onto a 384 SpectroCHIP II microarray and genotyping was performed on the MassArray platform (SEQUENOM, San Diego, CA, USA). The samples were assessed using the single-nucleotide polymorphisms of rs1061170 (Y402H) in the complement factor H (CFH) gene and rs10490924 (A69S) in the age-related maculopathy susceptibility 2 (ARMS2) gene.

**Statistical Analysis**

For all analyses, RPD were considered to be present in an eye only when detected by both NIR and SD-OCT, and a participant was considered to have RPD if they were present in either eye. The comparison of the laterality of RPD was performed using a one-sample binomial test. Receiver operating curves were plotted to determine sensitivity and specificity of CFP and FAF imaging for detecting RPD when compared with detection by NIR and SD-OCT combined, using the optimal cutoff with the highest likelihood ratio (sensitivity/1-specificity); this was performed for right and left eyes separately, due to the high level of intereye correlation. Binary logistic regression models using generalized estimating equations were used to account for the within-subject correlations (between eyes) when examining whether pigmentary abnormalities were present more often in eyes with RPD. These models were also used to examine the association between pigmentary abnormalities and RPD with the presence of atrophic changes. Age of the participants with and without RPD was compared using an independent sample t-test, and the distribution of sex was compared using a Pearson \( \chi^2 \) test, calculating the significance level based on the exact distribution of the test statistic. A binary logistic regression model was used to examine the interaction between age and sex to determine whether there was a significant difference in sex distribution with age. Differences in the distribution of parameters for smoking history, cardiovascular factors, and the distribution of the \( CFH \) and \( ARMS2 \) genotypes and minor allele frequency were examined using a Pearson \( \chi^2 \) test.
calculating the significance level based on the exact distribution of the test statistic. Adjustments for age and sex were also performed on all these parameters using binary or multinomial logistic regression analyses.

**RESULTS**

A total of 300 consecutive participants with bilateral large drusen that met the inclusion criteria were included in this study; 8 participants were excluded from this study because they did not meet the criteria of having good image quality on all imaging modalities. These participants were on average 70.6 ± 8.6 years of age (range, 50–92 years), and 217 (72.3%) were female.

### Prevalence and Detection of RPD

Reticular pseudodrusen were present on both SD-OCT and NIR in 169 (28.2%) eyes of 87 (29.0%) participants with bilateral large drusen, and occurred more often bilaterally (in 82 or 94% of these participants) than unilaterally (in 5 or 6% of these participants; \( P < 0.001 \)). Compared with the detection of RPD using both SD-OCT and NIR imaging, the sensitivity and specificity of CFP and FAF imaging for detecting RPD are shown in Table 1 for right and left eyes; CFP and FAF correctly detected RPD in 42% and 89% of eyes (71 and 151, respectively, of 169 eyes). Note that the participants who had RPD correctly detected on CFP were significantly younger than participants in whom it was missed (74.9 ± 6.7 and 78.1 ± 5.7 years, respectively; \( P = 0.022 \)), and this was the same case for FAF (72.2 ± 6.2 and 76.8 ± 6.5 years, respectively; \( P = 0.021 \)).

Examples of the detection of RPD using these imaging modalities are shown in Figure 1. The first example (Fig. 1A) illustrates how RPD were detected on all imaging modalities (CFP, FAF, NIR, and SD-OCT), but the second example (Fig. 1B) illustrates how RPD were missed on CFP, but detected on all the other imaging modalities (FAF, NIR, and SD-OCT).

### Demographic, Environmental, and Genetic Associations

Participants with RPD were on average older than participants without (mean ± SD, 76.2 ± 6.5 years and 68.4 ± 8.3 years, respectively, \( P < 0.001 \); Fig. 2), although no significant difference in sex distribution was observed in those with and without RPD (67% and 75% female, respectively; \( P = 0.200 \)). There was also no significant interaction between age and sex (\( P = 0.865 \)), indicating that there was no significant difference in sex distribution according to age.

There were no significant differences in smoking history (\( P = 0.306 \)), or diagnosis of hypertension, atherosclerosis, and hypercholesterolemia (\( P > 0.175 \)) between participants with and without RPD, even after adjusting for age and sex (\( P > 0.141 \)). No significant differences were also found for the distribution of the genotypes (even after adjusting for age and sex) or minor allele frequencies of the Y402H risk variant of the \( CFH \) gene between participants with and without RPD (\( P > 0.406 \)). However, significant differences were found in both the distribution of the genotypes (even after adjusting for age and sex) and minor allele frequencies of the A69S variant of the \( ARMS2 \) gene between participants with and without RPD (\( P < 0.008 \); Table 2). After adjusting for age and sex, the odds ratio of a participant having RPD was 3.38 (95% confidence interval [CI] 1.61–6.99; \( P = 0.001 \)) for the GT (\( \div \)) genotype and 8.54 (95% CI 3.44–21.23; \( P < 0.001 \)) for the TT (\( –/– \)) genotype compared with the GG genotype (\( +/- \)).

### Clinical Associations With RPD

Pigmentary abnormalities were present in 93 (55%) of 169 eyes with RPD and 206 (48%) of 431 eyes without RPD, and were not significantly different between the two groups even after adjusting for age (\( P > 0.112 \)). However, atrophic changes (including nascent geographic atrophy and drusen-associated atrophy detected on SD-OCT) were present in 33 (20%) of 169 eyes with RPD and 42 (10%) of 431 eyes without RPD, with RPD and pigmentary abnormalities both being independently associated with the presence of atrophic changes (\( P < 0.045 \); Table 3).

### DISCUSSION

This was the first prospective study to date to examine the prevalence and detection, as well as clinical, demographic, environmental, and genetic associations of RPD in participants with bilateral large drusen (considered to be intermediate AMD) using multimodal imaging. These characteristics are important for the clinical management of patients with intermediate AMD, and may have potential implications for clinical studies evaluating novel interventions.

Population-based studies have previously reported the prevalence of RPD to be between 0.7%\(^5\) and 1.95%\(^18\). These studies have defined RPD as the presence of confluent drusen forming an interlacing ribbon-like network solely on CFP (and not newer imaging modalities like NIR and SD-OCT), and did not include in the definition the appearance of an orderly array of relatively white, dot-like accumulations, which a recent study has shown to also correspond with RPD.\(^1\) In our study, in which only participants with bilateral large drusen were included, we found that RPD were present in more than a quarter of participants (29%) when using NIR and SD-OCT imaging combined, with a large majority of all participants having RPD bilaterally. Of all the eyes in which RPD were detected on both NIR and SD-OCT imaging, RPD were considered to be present in only 42.1% and 89.3% of these eyes when using CFP and FAF imaging, respectively. This is consistent with previous findings that reported that CFP is poorly sensitive at detecting RPD.\(^3,8,17\) Interestingly, participants with RPD who were missed on CFP and FAF imaging were significantly older than participants in whom it was correctly detected. Although this study included only participants who had good-quality images for all imaging modalities, this observation may be a result of the increased degree of preretinal absorption with increasing age, although we cannot confirm this as the lens status and grading were not recorded.

The rate of bilateral RPD found in this study (in 94% of participants) was also higher than those reported in two prior epidemiologic studies, which reported a prevalence of 59% to 65% and a 15-year cumulative incidence of 58% to 60%.\(^5,18\) These differences observed are most likely due to the different imaging modalities used to detect RPD. Our study examined the prevalence of RPD in persons with large drusen in both
eyes (intermediate AMD) with multimodal imaging, whereas those previous epidemiologic studies included all participants (with any stage of AMD, or without any features of AMD) and relied on only CFP. Instead, a more recent study that included NIR and SD-OCT for detecting RPD reported that RPD were bilateral in 98% of participants when the presence of RPD was identified in either eye. Although it is difficult to make a direct comparison with this study because it included any participant (either with any stage of AMD, or even without any features of AMD), these estimates are in much closer agreement with the findings of this study.

Although all participants in our study met the definition of intermediate AMD on CFP when using a recently proposed clinical classification system, atrophic changes including nGA and/or drusen-associated atrophy detected on SD-OCT were still present. In this study, the presence of RPD was found to be independently associated with these atrophic changes. Although we did not previously find RPD to be associated with the presence of nGA alone, reanalysis of our data to include drusen-associated atrophy detected on SD-OCT did indeed show that the presence of RPD were independently associated with the presence of these atrophic changes (data not shown).

**Figure 1.** Examples of RPD detected using CFP, FAF, NIR, and SD-OCT scans; an SD-OCT B-scan taken through an area with RPD is shown at the bottom in each example. The first example (A) illustrates how RPD were graded as being present on all imaging modalities, but the second example (B) illustrates how RPD were not graded as being present on CFP, but graded as being present on all the other imaging modalities.
This is in agreement with recent findings that the presence of RPD was an independent risk factor for the development of atrophic changes in the fellow eyes of individuals with unilateral CNV. In our cohort consisting of intermediate AMD participants with bilateral large drusen, those with RPD were significantly older than those without. However, no significant difference in sex distribution between these participants was observed. Although a direct comparison with existing literature cannot be made, two previous population-based studies have also observed an increased prevalence and incidence of RPD with age.5,18 A prospective study that compared participants with RPD only (without any drusen) and drusen only (without any RPD) also observed that participants with RPD only, were significantly older.24 These three studies also observed that RPD were significantly associated with the female sex,5,18,24 which our study did not observe; these differences may have been due to different recruitment biases in the different studies.

The lack of significant differences between participants with and without RPD (but with bilateral large drusen) for environmental factors, including smoking status, cardiovascular factors in our study are in agreement with previous studies that defined the presence of RPD using NIR and SD-OCT imaging, although with a different AMD disease severity.5,24–26 In contrast, previous population-based studies reported that current smoking at baseline was associated with the 15-year incidence of RPD detected on CFP, although other cardiovascular factors were not.5,18 This discrepancy in observations may again be due to the limitation of identifying RPD on CFP, and also the differences in the characteristics of the cohort examined.

We also examined the distribution of the genotypes at the two major AMD risk loci, CFH Y402H and ARMS2 A69S, in this cohort of participants with bilateral large drusen, and observed that the distribution was different for the ARMS2 gene, with a greater frequency of the minor allele for individuals with RPD than those without. Examining individuals with a different severity of AMD, three previous studies observed an association between RPD and ARMS2 polymorphisms,25–27 although two others did not.24,28 Although NIR and SD-OCT imaging were used to define RPD in these studies, the lack of agreement may again be due to the difficulty of identifying RPD on CFP, and also the differences in the characteristics of the cohort examined.

Although the function of the ARMS2 gene is not yet completely understood, the presence of this risk variant has been reported to confer an increased risk of developing advanced AMD.29,30 The greater

### Table 2. Comparison of the Demographic, Environmental, and Genetic Parameters in Participants With and Without RPD in Either Eye

<table>
<thead>
<tr>
<th></th>
<th>RPD, n = 87</th>
<th>No RPD, n = 87</th>
<th>P</th>
<th>P Adjusteda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>76.2 ± 6.5</td>
<td>68.4 ± 8.3</td>
<td>&lt; 0.001‡</td>
<td></td>
</tr>
<tr>
<td>Sex, female</td>
<td>58 (67)</td>
<td>159 (75)</td>
<td>0.200</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present or previous</td>
<td>34 (39)</td>
<td>98 (46)</td>
<td>0.306</td>
<td>0.213</td>
</tr>
<tr>
<td>Never</td>
<td>53 (61)</td>
<td>115 (54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>41 (47)</td>
<td>94 (44)</td>
<td>0.702</td>
<td>0.141</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>11 (13)</td>
<td>15 (7)</td>
<td>0.173</td>
<td>0.710</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>37 (43)</td>
<td>87 (41)</td>
<td>0.897</td>
<td>0.564</td>
</tr>
<tr>
<td>CFH risk allele (rs1061170)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (−/−)</td>
<td>15 (17)</td>
<td>34 (16)</td>
<td>0.607</td>
<td>0.531</td>
</tr>
<tr>
<td>CT (±)</td>
<td>42 (48)</td>
<td>92 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (+/+)</td>
<td>30 (35)</td>
<td>87 (41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor allele (C) frequency</td>
<td>102 (59)</td>
<td>266 (62)</td>
<td>0.406‡</td>
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</tr>
<tr>
<td>ARMS2 risk allele (rs10490924)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (−/−)</td>
<td>19 (22)</td>
<td>80 (38)</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GT (±)</td>
<td>44 (51)</td>
<td>100 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (+/+)</td>
<td>24 (28)</td>
<td>31 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor allele (T) frequency</td>
<td>92 (53)</td>
<td>166 (39)</td>
<td>0.002‡</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as n (%) or mean ± SD and analyzed using a Fisher’s Exact Test unless otherwise indicated. Bold numbers indicate statistically significant at P < 0.05.

* Adjusted for age and sex in a binary or multinomial logistic regression analysis.
† Independent samples t-test.
‡ Pearson χ² test for its exact counterpart.
frequency of the ARMS2 risk allele in individuals with bilateral large drusen and RPD in either eye therefore further implicates RPD as a risk factor for progression toward advanced AMD, as suggested by findings to date.3-5 This finding also suggests that a common mechanism between the ARMS2 gene and RPD may be present, with further investigations required to elucidate this.

The findings of this study have important implications for the management of intermediate AMD patients. Of note, RPD were present in up to 29% of participants with bilateral large drusen, with more than half of these participants missed if CFP alone was used to detect their presence. Participants with RPD also had a greater frequency of the ARMS2 risk allele, which further supports the suggestion that RPD confers an increased risk of progression to advanced AMD.3-5 Atrophic changes detected on SD-OCT, but not on CFP, were also present more often in eyes with RPD. Collectively, these findings underscore the importance of imaging modalities such as SD-OCT, NIR, and FAF for complete characterization and counseling of those with intermediate AMD, particularly through the detection of RPD. In an era in which interventional studies are being conducted in the early stages of AMD, future studies may also need to consider the presence of RPD when allocating participants into different treatment groups.

Strengths of this study include its prospective nature, large sample size, and careful characterization of the participants with high-quality imaging. In addition, only participants with bilateral large drusen were included in this study, making it a comprehensive study of this high-risk group.3-6,10 However, limitations of this study include the cross-sectional design and having only a single grader perform all the image grading. First, the cross-sectional nature of this study renders it susceptible to prevalence-incidence bias,31 whereby associations between RPD and mortality rate may influence the demographic or environmental factors, for example. The participants included in our study were also referred by their eye care providers for consideration of participation in clinical studies being conducted at our research unit, and may not be representative of the entire population of intermediate AMD. Second, subjective grading of all imaging modalities by a single grader may be less precise as compared to having two independent graders. However, the grader was experienced and masked to all imaging modalities, and we therefore believe the findings of this study are still valid.

In conclusion, RPD were present in more than a quarter of patients with bilateral large drusen, in whom more than half the eyes with RPD would have been missed if CFP were solely used to detect their presence. Atrophic changes detected on SD-OCT were more likely to be present in eyes with RPD, and participants with RPD had a greater frequency of the ARMS2 risk allele than those without. These findings are important to consider in the clinical management of patients with intermediate AMD, and in the design of studies aiming to slow progression to advanced AMD.

### References


