Reticular Pseudodrusen Are Associated With More Advanced Para-Central Photoreceptor Degeneration in Intermediate Age-Related Macular Degeneration

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PURPOSE. The purpose of this study was to examine retinal topographical differences between intermediate age-related macular degeneration (iAMD) with reticular pseudodrusen (RPD) versus iAMD without RPD, using high-density optical coherence tomography (OCT) cluster analysis.

METHODS. Single eyes from 153 individuals (51 with iAMD+RPD, 51 with iAMD, and 51 healthy) were propensity-score matched by age, sex, and refraction. High-density OCT grid-wise (60 × 60 grids, each approximately 0.01 mm² area) thicknesses were custom-extracted from macular cube scans, then compared between iAMD+RPD and iAMD eyes with correction for confounding factors. These “differences (μm)” were clustered and results de-convoluted to reveal mean difference (95% confidence interval [CI]) and topography of the inner retina (retinal nerve fiber, ganglion cell, inner plexiform, and inner nuclear layers) and outer retina (outer plexiform/Henle’s fiber/outer nuclear layers, inner and outer segments, and retinal pigment epithelium-to-Bruch’s membrane [RPE-BM]). Differences were also converted to Z-scores using normal data.

RESULTS. In iAMD+RPD compared to iAMD eyes, the inner retina was thicker (up to +5.89 [95% CI = +2.44 to +9.35] μm, \( P < 0.0001 \) to 0.05), the outer para-central retina was thinned (up to −3.21 [95% CI = −5.39 to −1.03] μm, \( P < 0.01 \) to 0.001), and the RPE-BM was thicker (+3.38 [95% CI = +1.05 to +5.71] μm, \( P < 0.05 \)). The majority of effect sizes (Z-scores) were large (−3.13 to +1.91).

CONCLUSIONS. OCT retinal topography differed across all retinal layers between iAMD eyes with versus without RPD. Greater para-central photoreceptor thinning in RPD eyes was suggestive of more advanced degeneration, whereas the significance of inner retinal thickening was unclear. In the future, quantitative evaluation of photoreceptor thicknesses may help clinicians monitor the potential deleterious effects of RPD on retinal integrity.

Keywords: reticular pseudodrusen (RPD), subretinal drusenoid deposits, age-related macular degeneration (AMD), anatomy, clustering, optical coherence tomography (OCT), spatial, retinal thickness

R eticular pseudodrusen (RPD; also known as sub-retinal drusenoid deposits) are sub-retinal granular extracellular deposits containing photoreceptor and retinal pigment epithelium (RPE) byproducts. RPD in the presence of age-related macular degeneration (AMD) is important to recognize as they may be associated with greater risk of progression to late AMD, although some studies have suggested otherwise. AMD with RPD has also been associated with faster progression of geographic atrophy, poorer response to AMD treatments and worse visual sensitivity when compared to AMD without RPD.

To better understand why the RPD phenotype may (or may not) confer worse outcomes in patients with AMD, several studies have investigated the effects of RPD on in vivo retinal anatomy and yielded conflicting results. For example, eyes with early and/or intermediate AMD (iAMD) with RPD have been found to have total retinal thickness both thinned or non-different compared to early/iAMD eyes without RPD. Similarly, inner retinal thickness has been reported to be thinned or non-different, and outer retinal thickness has been reported to be thinned, thickened, or non-different in early/iAMD eyes with RPD versus without RPD.

Dissimilarities in study characteristics, such as varying population demographics and methods of RPD identification/grading, may explain some of the discrepancies.
in the aforementioned studies. However, more notably, all these studies make the a priori assumption that retinal changes will follow an existing spatial template, such as predefined points, the Early Treatment Diabetic Retinopathy Study (ETDRS) sectors, or large/global areas. These arbitrary spatial groupings introduce statistical bias known as the modifiable areal unit problem (MAUP), whereby results may be misrepresented or even masked depending upon how data are spatially grouped (e.g., via the aforementioned spatial templates).

Recently, we addressed the MAUP by sampling the macula using 3600 or 60 × 60 OCT grids across each individual retinal layer. These grids could not be further meaningfully divided, resulting in data sets of nonmodifiable high-density grid-wise retinal layer thicknesses. To avoid any a priori spatial grouping of data which would induce the MAUP, we then applied unsupervised cluster analysis post hoc to classify grids into clusters that were statistically similar within-cluster and statistically separable between clusters. This method revealed in vivo retinal anatomical topographies of AMD and normal aging eyes that have otherwise been undetectable via arbitrary spatial groupings, such as the ETDRS sectors. For example, using this method, we found extensive spatial patterns of thinning with large effect sizes across each individual retinal layer, suggesting that retinal-spanning degenerative changes begin at the early stage of AMD.

Thus, in this study, we aimed to use high-density optical coherence tomography (OCT) cluster analysis to resolve the MAUP and clarify the potential effects of RPD on retinal topography in iAMD. We hypothesize that RPD presence is associated with greater thinning in iAMD eyes, particularly at the photoreceptor layers. These results could help answer whether RPD is associated with more advanced degeneration in iAMD and improve understanding of where in the retina RPD may impact, thus guiding clinical monitoring and future intervention protocol.

**Methods**

**Study Population**

The study population was recruited through retrospective review of all patient records from the Centre for Eye Health (CFEH) Sydney, Australia, from January 1, 2009, to December 31, 2021. CFEH is a referral-based clinic with advanced diagnostic testing and management of eye disease by optometrists and ophthalmologists. All patients who had their records reviewed had provided prior written informed consent for research use of their de-identified data approved by the Biomedical Human Research Ethics Advisory Panel of the University of New South Wales and in accordance with the Declaration of Helsinki.

All participants regardless of study group were required to be ≥ 50 years of age and have no macular-involving disease or significant structural abnormality (except RPD and/or iAMD for the respective study groups). Optic neuropathies (including suspected or confirmed glaucoma) were excluded due to partial macular involvement. All diagnoses were formed by two non-blind CFEH clinicians (optometrists and/or ophthalmologists) and confirmed by investigator author Matt Trinh. A single eye was selected for each participant, and simple randomization was used if both eyes were eligible. Systemic factors, including self-reported presence of diabetes mellitus (type 1 or 2), smoking (ever or never), and hypertension, which have some (conflicting) evidence of association with retinal thicknesses, were compared between groups after propensity-score matching. These factors were not included in propensity-score matching to preserve sample size, considering increased prevalence of these systemic factors with age.

Eligibility of iAMD-RPD eyes required the presence of RPD based on the study by Ueda-Arakawa et al. (i.e., ≥ 5 hyper-reflective lesions above the RPE via OCT, and iAMD-based on a modified Beckman Initiative classification; i.e., presence of large drusen ≥ 125 μm or pigmentary abnormalities related to AMD with at least medium drusen via color fundus photography). Other imaging modalities, such as color fundus photography, near-infrared, and fundus autofluorescence, were available for all patients screened for eligibility as part of standard CFEH clinical care and used to aid diagnoses and exclude other ocular pathology. Participants aged 50 to 54 years were included if iAMD phenotypic criteria were fulfilled in concordance with other notable studies. Eligibility of iAMD eyes required the presence of iAMD without RPD. Eligibility of normal healthy eyes required visual acuity better than 0.1 logMAR for participants < 60 years or 0.2 logMAR for participants ≥ 60 years, the latter criteria being more lenient to account for normal age-related decline of visual acuity from numerous factors, including cataracts.

**Propensity-Score Matching**

The iAMD-RPD eyes were propensity-score matched to iAMD eyes and then normal eyes using multi-variable logistic regression considering age, sex, and spherical equivalent refraction as co-variables. Fuzzy matching without replacement was performed to randomize selection of participants, whereas mitigating imbalance of potential confounders between groups, as opposed to exact matching which can significantly reduce sample size in a limited sampling pool and increase selection bias. Match tolerance of propensity scores was increased after iterative random draws until maximal sample size.

**Image Acquisition and Retinal Layer Segmentation**

OCT macular cube scans across an area of 30 × 25 degrees or approximately 8640 × 7200 μm (width × height) were acquired from the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). The width was parallel to the axis between the fovea-to-optic nerve head center and the height perpendicular to this width. All conversions between retinal μm and degrees were performed under the model approximation that 1 degree = approximately 288 μm which maintains a relatively linear relationship within the retinal (non-peripheral) area analyzed in this study. The scanning protocol contained 61 B-scans spaced approximately 118 μm apart, which was the highest number of B-scans commercially available for the Spectralis SD-OCT without significant compromise in image quality. If multiple scans per participant were eligible for inclusion, the latest scan above 15 dB signal strength and without significant artifacts was selected. Ocular tilt, automatic segmentation, and then manual correction of segmentation were applied.
to each scan via the HRA/Spectralis Viewing Module 6.9.5.0 (Heidelberg Engineering).

As previously described, each participant was randomized into one of two blocks, and each block had their selected OCT macular cube scans independently reviewed by two observers (authors M.T. and N.E.). Retinal layer segmentation for the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL)/Henle’s fiber layer (HFL)/outer nuclear layer (ONL), IS/OS, and RPE-Bruch’s membrane (BM) were manually corrected where necessary (Fig. 1A, pink insert). Due to inconsistent reflectivity with Henle’s fibers in the OPL/HFL/ONL, disrupted photoreceptor ellipsoid in the IS/OS, particularly in diseased eyes, these layers were combined from their respective individual layers. The alternate block was then reviewed by authors Matt Trinh and Natalie Eshow, and further manual correction performed after discussion and consensus. There was non-blinding to study group as drusen and/or pigmentary changes are obvious during the segmentation process. Manual correction of segmentation was used as the “ground-truth” for retinal anatomy in concordance with other studies and has demonstrated excellent repeatability and reproducibility for AMD eyes.

Segmentations were manually corrected to continue through (rather than around) large vessels to mitigate effect on thicknesses (see Fig. 1A, asterisk). Segmentations were also manually corrected around conventional drusen and RPD (see Fig. 1A, black arrowhead). Regarding RPD, the middle of the external limiting membrane was segmented to continue through RPD apices (for RPD significantly protruding toward the inner retina) and the RPE was segmented to continue under RPD (see Fig. 1A, white arrowheads), in correspondence with histology.

High-density OCT Thickness Comparison Between iAMD+RPD and iAMD Eyes

Custom-extraction of thicknesses was executed using MATLAB (version 9.9; MathWorks, Natick, MA, USA) code developed by investigator author David Alonso-Caneiro and applied to OCT macular volume RAW and XML files with adjustment for foveal location and foveal-to-optic nerve head tilt. This code enabled sampling and averaging of the volumetric OCT dataset into 3600 grids (60 × 60 grids, each approximately 0.01 mm² area, comprised of or 0.4 × 0.4 degrees or approximately 115 × 115 μm sides; i.e. thicknesses, foveally centered and covering 24 × 24 degrees or approximately 6912 × 6912 μm; Fig. 1B) across each retinal layer. Grid density of 60 × 60 was utilized to maximize coverage of the 61 B-scans per macular cube scan. Grid coverage of approximately 6912 × 6912 μm was in accordance with the default commercially available Spectralis Viewing Module grid coverage for 64 grids (8 × 8 grids, each approximately 0.75 mm² area). Note that grid coverage (24 × 24 degrees or approximately 6912 x 6912 μm) did not extend to the entire scan area (30 × 25 degrees or approximately 8640 × 7200 μm), but did include the entire macula (approximately 19 degrees diameter or approximately 5500 μm).

Grid-wise thicknesses of iAMD+, iAMD, and normal groups across each retinal layer were used to develop multi-variable linear regression models enabling correction for potential confounding from age, sex, refraction, presence of pigmentary abnormalities, and average RPE-BM thickness. Refraction was used as a proxy for axial length based on strong correlation between the two variables. As a constituent of magnification correction factor, the correction (and matching) of refraction also ensured that between-group analyses maintained statistical robustness regardless of within-group differences in magnification factors. Average RPE-BM thickness was included to account for differing drusen load between groups (except when comparing the RPE-BM between groups).

Grid-wise thicknesses of an iAMD+RPD eye would be subtracted by grid-wise thicknesses of an iAMD eye corrected to the same age, sex, refraction, presence of pigmentary abnormalities, and average RPE-BM thickness, resulting in grid-wise difference (μm; see Fig. 1B). This process was repeated for all 3600 grids, for each retinal layer, and for all participants to account for potential confounding at the grid-wise level (pre-clustering) rather than the group level (post-clustering). This ensured that regression characteristics were not indiscriminately applied to all grids within each spatial group (cluster) which could otherwise introduce statistical bias known as the MAUP (i.e. the potential misrepresentation of results dependent upon how data are spatially grouped and analyzed).

Clustering and Retinal Topography Between iAMD+RPD and iAMD Eyes

Grid-wise differences (μm) between iAMD+RPD and iAMD eyes were assigned into clusters which represented groupings of statistically similar values within clusters and statistically separable values between clusters. Unsupervised cluster analysis was performed using the two-step algorithm due to its robustness compared to several other cluster algorithms. Grid order was randomized and a log-likelihood method applied with consideration of the lowest Bayesian Information Criterion, intra-cluster similarity, and inter-cluster separability to generate clusters for each individual retinal layer. The clustering process was reiterated until all clusters were separable by >95th percentile mean difference limits, ensuring that all clusters were significantly different (P < 0.05) from each other (Fig. 1C). Note that cluster confidence intervals (CIs) can overlap and still be significantly different, as separability by >95th percentile mean difference limits is approximately equal to separability of 84th percentile limits around individual means.

Clusters were then de-convoluted to generate means (95% CI) displayed graphically (Fig. 1D, left) and patterns displayed topographically (see Fig. 1D, right). Clusters were labeled positive or negative then ranked based on magnitude of difference (μm; see Fig. 1D, middle legend). Differences were also converted to Z-scores (i.e. SD units from normal [corrected]), to demonstrate effect sizes. Non-zero cluster proportional areas (%) were reported to justify use of and compare descriptive spatial delineations for quadrants and centrality. Quadrants were defined as: superior, nasal, inferior, and temporal. Centrality was defined (anatomically) as: “central macula” (approximately 5.2 degrees or approximately 1500 μm diameter), “peri-central macula” (approximately 7 degrees or approximately 2000 μm diameter ring), “para-central macula” (approximately 12.2 degrees or approximately 3500 μm diameter ring), “peripheral macula” (approximately 19 degrees or approximately 5500 μm diameter ring), and “extra-macula” (outside approximately 19 degrees or approximately 5500 μm diameter; see Fig. 1D,
Retinal Topography of iAMD With Versus Without RPD

FIGURE 1. OCT macular cube scans were automatically segmented then manually corrected to define individual retinal layers (pink insert) in each B-scan using the HRA/Spectralis Viewing Module (A). Notable segmentation corrections included continuing through (rather than around) large vessels (black asterisk) and resolving mis-segmentation around conventional drusen and RPD (black arrowhead). In particular, the external limiting membrane was segmented to continue through RPD apices and the RPE was segmented to continue under RPD (white arrowheads). High-density grid thicknesses were then custom-extracted for each retinal layer across 3600 (60 × 60) grids foveally centered and covering 24 × 24 degrees or approximately 6912 × 6912 μm (B). Grid-wise thicknesses between iAMD+RPD and iAMD eyes were compared with correction for co-variables, and resultant grid-wise thickness differences simply denoted as “differences (μm).” Within each retinal layer, Two-Step clustering was applied to differences and the process re-iterated with a smaller cluster size until all clusters were separable by >95th percentile mean difference limits (C). Clusters were then de-convoluted to generate means [95% CI] displayed graphically (left), and patterns displayed topographically (right) (D). Clusters were labelled positive or negative then ranked based on magnitude of difference (μm): $C_{+1}, +2, +3… =$ increasingly thicker = darker green; $C_{-1}, -2, -3… =$ increasingly thinner = darker blue. $C_0 =$ within 95th percentile distribution limits from zero = cream. The topography (right) shows an example of thickened central and peripheral macula, and thinned para-central macula. Grey cross denotes foveal center, dotted grey lines denote central macula (approximately 5.2 degrees or approximately 1500 μm diameter), peri-central macula (approximately 7 degrees or approximately 2000 μm diameter ring), para-central macula (approximately 12.2 degrees or approximately 3500 μm diameter ring), peripheral macula (approximately 19 degrees or approximately 5500 μm diameter ring), and extra-macula (outside approximately 19 degrees or approximately 5500 μm diameter ring). Corresponding scales bottom right and all images in right eye format. Note the fundus photographs are examples used for orientation purposes. Abbreviations: RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL/HFL/ONL outer plexiform, Henle’s fiber, and outer nuclear layers; IS/OS, inner and outer segments; RPE-BM, retinal pigment epithelium-to-Bruch’s membrane.
right dotted grey lines). These terms were applied to each individual retinal layer excluding the RNFL due to its non-concentric distribution. The RNFL was spatially delineated according to macula quadrants only, particularly as “peri-papillary/para-papillary” are not well defined.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism version 9.3.1, SPSS Version 25, and Microsoft Excel Version 2203. Statistical significance was considered as $P < 0.05$. Continuous values were expressed as mean (95% CI) unless otherwise specified. Non-continuous variables, for example, sex, presence of pigmentary abnormalities, and study group, were dummy-coded for regression analyses. Normality was assessed using the D’Agostino-Pearson test. Thicknesses (by grid or cluster) were derived from each participant as a single unit of observation (i.e. independent data). Hence, single comparisons of continuous data between groups used unpaired Student’s $t$-test or Mann-Whitney $U$ test depending on normality. As each comparison was individually important, there was no statistical adjustment for multiple comparisons (within each individual retinal layer) and instead results were considered contextually. Multiple comparisons of continuous data between groups used one-way ANOVA and Tukey’s multiple comparisons test. Comparisons of continuous data within groups (i.e. between clusters within each individual retinal layer) used paired $t$-test or mixed-effects repeated measures model with non-continuous variables, for example, age, sex, presence of pigmentary abnormalities, and study group, were considered contextually. Comparisons of categorical data used Chi-squared ($\chi^2$) test for independence. Resultant grid-wise differences were classified into clusters, and separability confirmed between all clusters within each individual retinal layer ($P < 0.0001$ all). All between-group comparisons of cluster zero ($C_0$) were non-different ($P > 0.05$). To demonstrate effect sizes, differences were also converted to Z-scores using normal data.

RESULTS

Participant Demographics

Single eyes from 153 individual participants were included in this study forming 3 groups: 51 with iAMD $\pm$ RPD, 51 with iAMD, and 51 normal healthy eyes. Following propensity-score matching, propensity scores (logistic regression predicted probability mean $\pm$ SD) were relatively balanced between the iAMD $\pm$ RPD and iAMD groups (0.52 $\pm$ 0.11 and 0.48 $\pm$ 0.08, respectively), and iAMD $\pm$ RPD and normal healthy groups (0.53 $\pm$ 0.14 and 0.47 $\pm$ 0.11, respectively). Consequently, there were no significant differences regarding age, sex, or spherical equivalent refraction between any group (Table 1). Comparison of the presence of systemic factors (i.e. diabetes mellitus, smoking, and hypertension), were also non-different between groups. Expectedly, presence of pigmentary abnormalities was different between groups, and average RPE-BM thickness was significantly greater comparing iAMD $\pm$ RPD to iAMD ($P < 0.05$) and iAMD $\pm$ RPD to normal ($P < 0.0001$), but not iAMD to normal ($P = 0.1$) groups. Thus, in addition to age, sex, and refraction, subsequent analyses also corrected for the presence of pigmentary abnormalities and average RPE-BM thickness.

Inner Retinal Topographical Differences Between iAMD $\pm$ RPD and iAMD Eyes

Grid-wise thicknesses across each retinal layer were compared between iAMD $\pm$ RPD and iAMD eyes with correction for confounding. Resultant grid-wise differences were classified into clusters, and separability confirmed between all clusters within each individual retinal layer ($P < 0.0001$ all). All between-group comparisons of cluster zero ($C_0$) were non-different ($P > 0.05$). To demonstrate effect sizes, differences were also converted to Z-scores using normal data.

RNFL grid-wise differences between iAMD $\pm$ RPD and iAMD eyes were classified into three clusters (Fig. 2A). In the iAMD $\pm$ RPD group, there was thicker RNFL ($C_{+1}$, $+1.87$ [95% CI $= +0.36$ to $+5.39$] $\mu$m, $P < 0.05$; $C_{+2}$, $+5.89$ [95% CI $= +2.44$ to $+9.35$] $\mu$m, $P < 0.01$) occupying 56.7% of the macular scan area (Fig. 2B; Table 2). Thicker RNFL was more evident at the superior than inferior ($C_{+1}$ proportional area, 64% vs. 52%, $P < 0.01$) and nasal than temporal ($C_{+1}$, 55% vs. 33%, $P < 0.05$; $C_{+2}$, 21% vs. 0%, $P < 0.0001$; Supplementary Table S1) quadrants. The central, inferior wedge, and part superior wedge macular scan areas (43.4%) were non-different ($C_0$) between iAMD eyes with versus without RPD. Z-scores (SD units from normal) revealed large effect sizes in $C_{+1}$, $+2$ (95% CI $= +1.04$ to $+1.28$).

GCL differences between iAMD $\pm$ RPD and iAMD eyes were assigned to two clusters (Fig. 2C). In the iAMD $\pm$ RPD group, there was thicker GCL ($C_{+1}$, $+2.37$ [95% CI $= +1.32$ to $+3.41$] $\mu$m, $P < 0.0001$) occupying 42.4% of the macular scan area and with large effect size of $+1.26$ (Fig. 2D, see Table 2).

### Table 1. Study Population Demographics

| Eyes, n | IAMD $\pm$ RPD | iAMD | Normal | $P$ Value |
|---------|-----------------|------|--------|-----------|
| Age, y  | 76.64 [74, 79.29] | 74.26 [72.06, 76.46] | 73.21 [71.62, 74.79] | 0.08$^1$ |
| Sex, F:M| 31:20           | 33:18 | 36:15 | 0.58$^1$ |
| Spherical equivalent refraction, diopters | 0.19 [–0.29, 0.67] | 0.59 [0.05, 1.12] | 0.63 [0.28, 0.98] | 0.35$^1$ |
| Diabetes mellitus, presence:absence    | 7:44       | 7:44       | 4:47       | 0.57$^1$ |
| Smoker, ever:never                      | 7:44       | 9:42       | 4:47       | 0.34$^1$ |
| Systemic hypertension, presence:absence | 24:27      | 25:26      | 27:24      | 0.83$^1$ |
| Pigmentary abnormalities, presence:absence | 18:33  | 20:31 | 0:51 | $<0.0001^1$ |
| Average RPE-BM thickness, $\mu$m        | 15.56 [14.47, 16.66] | 14.25 [13.53, 14.97] | 13.11 [12.78, 13.45] | $<0.0001^1$ |

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1 One-way ANOVA and $^2$ $\chi^2$ test for comparison between the three groups. Average RPE-BM thickness was significantly different comparing iAMD $\pm$ RPD to iAMD ($P < 0.05$) and iAMD $\pm$ RPD to normal ($P < 0.0001$), but not iAMD to normal ($P = 0.1$) groups.
Figure 2. Retinal topography between iAMD_1 and iAMD groups in the inner retina. Between-groups cluster differences presented graphically for the (A) RNFL, (C) GCL, (E) IPL, and (G) INL. Significance above each data point derived from unpaired Student’s t-tests or Mann-Whitney U tests: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Z-scores below the x-axis. Between-groups cluster differences then presented topographically for the (B) RNFL, (D) GCL, (F) IPL, and (H) INL. Dotted grey lines for the RNFL denote quadrants. Presentation as in Figure 1D.
There were no quadrant biases, although thicker GCL was most evident at the peri-central macula (C_1, 93.1%, P < 0.0001; see Supplementary Table S1). IPL differences were also classified into two clusters (Fig. 2E) with thicker IPL (C_1, +1.71 [95% CI = +0.98 to +2.45] μm, P < 0.0001) occupying 40.8% macular scan area with large effect size (+1.35; Fig. 2F, see Table 2) in the iAMD_{RPM} group. There were no quadrant biases, although thicker IPL was most evident at the peri-central macula (C_1, 80%, P < 0.0001; see Supplementary Table S1).

Finally, the INL differences were assigned to two clusters (Fig. 2G) with thicker INL (C_1, +1.72 [95% CI = +1.05 to +2.39] μm, P < 0.0001; 41.4% macular scan area) with large effect size (+1.91; Fig. 2H, see Table 2) in the iAMD_{RPM} group. There were no quadrant biases, although thicker INL was most evident at the central macula (C_1, 64%, P < 0.05; see Supplementary Table S1).

### Table 2. Cluster Differences for iAMD_{RPM} – iAMD Eyes

| Cluster | INL Cluster sizes /3600 per layer (%) | iAMD_{RPM} – iAMD | Effect size (Z-score) | P-value |
|---------|--------------------------------------|-------------------|----------------------|---------|
| RNFL    | 0                                    | 1561 (43.4%)      | -0.06 [-0.26, 0.73]  | 0.13    |
|         | +1                                   | 1666 (46.3%)      | +1.87 [0.36, +3.39]  | +1.04   |
|         | +2                                   | 373 (12.0%)       | +5.89 [2.44, +9.35]  | +1.28   |
| GCL     | 0                                    | 2075 (57.6%)      | 0.39 [-0.21, +0.99]  | 0.35    |
|         | +1                                   | 1525 (42.4%)      | +2.37 [1.32, +3.41]  | +1.26   |
| IPL     | 0                                    | 2130 (59.2%)      | +0.16 [-0.45, +0.77] | 0.15    |
|         | +1                                   | 1470 (40.8%)      | +1.71 [0.98, +2.45]  | +1.35   |
| INL     | 0                                    | 2108 (58.6%)      | -0.3 [-0.34, +0.94]  | 0.28    |
|         | +1                                   | 1492 (41.4%)      | +1.72 [1.05, +2.39]  | +1.91   |
| OPL/HFL/ONL | -1                                  | 989 (27.5%)      | -3.21 [-5.39, -1.03] | -1.24   |
|         | 0                                    | 2611 (72.5%)      | -0.47 [-2.09, +1.16] | -0.22   |
| IS/OS   | -1                                   | 759 (21.1%)       | -1.67 [-2.69, -0.66] | -1.13   |
|         | 0                                    | 2841 (78.9%)      | +0.12 [-0.59, +0.83] | 0.27    |
| RPE-BM  | -1                                   | 2510 (69.7%)      | +0.57 [+0.16, +1.39] | 0.61    |
|         | +1                                   | 1050 (30.3%)      | +3.38 [+1.05, +5.71] | +1.24   |

Differences expressed as mean difference [95% CI] μm. Cluster sizes expressed as grid counts /3600 per layer (%). Significance denoted by: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

### Discussion

High-density OCT cluster analysis of iAMD eyes with RPD versus without RPD unveiled anatomic topographical differences with large effect sizes in each retinal layer. Thicker inner retina (RNFL, GCL, IPL, and INL) in iAMD_{RPM} compared to iAMD may be related to inner retinal remodelling.90–93 Meanwhile, thinner photoreceptor layers (OPL/HFL/ONL and IS/OS) para-centrally suggested that RPD are associated with more advanced retinal degeneration. In addition, thicker RPE-BM despite propensity-score matching for age, sex, and refraction highlighted the need for studies to correct for AMD severity when assessing RPD. These results implicate RPD as a significant associative factor to more advanced retinal degeneration in iAMD eyes at least in the para-central outer retina, and direct attention toward specific retinal areas that RPD may impact for closer clinical monitoring and future quantitative evaluation.

### Partial Evidence for Inner Retinal Remodeling

The inner retinal layers (RNFL, GCL, IPL, and INL) were thicker in iAMD_{RPM} compared to iAMD eyes, consistent with other studies.24,25,32,34,35 Our secondary analysis helped to contextualize these results and revealed significant RNFL thinning and significant GCL, IPL, and INL thickening of iAMD_{RPM} from normal eyes. Interestingly, this re-affirms recent theory that inner retinal thickening (GCL, IPL, and INL) which is “sandwiched” between contemporaneous RNFL and photoreceptor degeneration (comparing iAMD_{RPM} to normal healthy eyes), may be part of the degenerative process.24,32,35 as seen with inner retinal remodeling (e.g. cellular hyperactivity and membrane hyperpermeability) in other outer retinal degenerations.90–92,95,96 A histological case study has demonstrated increased glial fibrillary acidic protein expression – a marker of retinal glial stress – at the inner retina overlying RPD, although to our knowledge no strong evidence of inner retinal remodeling associated with RPD has yet been reported. Our findings
of peri-central inner retinal thickening when comparing iAMD_{RPD} to both iAMD and normal healthy eyes aligns with the greater density of inner retinal cells including ganglion cells, bipolar cells, horizontal cells, amacrine cells, and Müller cells, although further study is required to explore inner retinal changes associated with RPD.
Figure 4. Retinal topography between iAMD + RPD and normal groups in the inner retina. Between-groups cluster differences presented graphically for the (A) RNFL, (C) GCL, (E) IPL, and (G) INL, and topographically for the (B) RNFL, (D) GCL, (F) IPL, and (H) INL. Presentation as in Figure 2.
Table 3. Cluster Differences for iAMD+RPD – Normal Eyes at the Inner Retina

| Clusters | Cluster sizes /3600 per layer (%) | Differences (μm) | Effect size (Z-score) |
|----------|-----------------------------------|------------------|----------------------|
| RNFL     | -1                                | -2.1 [-3.7, -0.5]* | -0.99 |
|          | 0                                 | +1.08 [-0.47, +2.64] | 0.57 |
| GCL      | 0                                 | -0.24 [-0.96, +0.47] | -0.18 |
|          | +1                                | +1.04 [+0.29, +1.79]** | +0.75 |
|          | +2                                | +2.82 [+1.67, +3.97]**** | +1.7 |
| IPL      | 0                                 | -0.32 [-0.96, +0.32] | -0.29 |
|          | +1                                | +1.22 [+0.55, +1.89]*** | +1.08 |
| INL      | 0                                 | -0.12 [-0.78, +0.55] | -0.11 |
|          | +1                                | +1.66 [+1.02, +2.31]**** | +1.9 |

Differences expressed as mean difference [95% CI] μm. Cluster sizes expressed as grid counts /3600 per layer (%). Significance denoted by: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Furthermore, why the inner retina may be altered in a primarily outer retinal disease is yet unknown. Previously, we considered the possibility of anterograde trans-synaptic degeneration but could not observe any significant relationships for OCT thickness changes between the outer and inner retinal layers in early/iAMD. Alternatively, there are suggestions that the outer and inner retinal layers in AMD could be linked via common inflammatory and/or vascular pathways, such as the choroid. Such theories could explain why AMD prevalence is greater in patients with glaucoma, for example, despite controlling for related variables such as age and sex.

RPD Are Associated With More Advanced Outer Retinal Degeneration

Recently, we found para-central photoreceptor thinning in early/iAMD compared to normal eyes, which corresponds to rod susceptibility in AMD seen via structural and functional measures. In this study, the RPD phenotype of iAMD presented with even greater photoreceptor thinning para-centrally, suggesting even greater rod susceptibility. These differences were unlikely to be an artifact of mechanical compression from underlying drusen as for each layer (except the RPE-BM), we corrected for differences in RPE-BM thickness between groups. Our previous study also confirmed photoreceptor thinning began at the early stage of AMD, prior to significant thinning of the RPE-BM. Photoreceptor thinning was concordant with the overwhelming number of studies demonstrating that RPD are associated with increased risk of progression to late AMD. Subanalyses that this para-central thinning was more prevalent superiorly in the para-central outer retinal layers and/or rods.

The primary limitation of this study is the use of cross-sectional OCT data, which does not identify specific cellular and synaptic changes nor their temporality (i.e. do RPD cause macular anatomic changes or are patients with specific macular anatomy more susceptible to developing RPD?). The latter theory has garnered increasing attention as recent studies confirm various AMD genotypes influence phenotypic expressions, such as retinal layer thicknesses even prior to the development of AMD. Regardless of the chronology of events, the association of RPD with more advanced para-central outer retinal degeneration remains a relevant consideration for closer monitoring of patients with iAMD.

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Retinal Topography of iAMD With Versus Without RPD

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