Published in final edited form as:

Am J Ophthalmol. 2017 February ; 174: 42–55. doi:10.1016/j.ajo.2016.10.005.

Choriocapillaris Non-Perfusion is Associated with Poor Visual Acuity in Eyes with Reticular Pseudodrusen

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Abstract

Purpose—To study choriocapillaris blood flow in age-related macular degeneration (AMD) using optical coherence tomography angiography (OCTA) and study its correlation to vision (VA) in eyes with reticular pseudodrusen (RPD) versus those with drusen without RPD (drusen).

Design—Cross-sectional study

Methods—Patients with either drusen or RPD in early AMD underwent OCTA imaging of the superior, inferior, and/or nasal macula. We quantified “percent choriocapillaris area of non-perfusion” (PCAN) in eyes with RPD versus those with drusen. We assessed the repeatability of PCAN and its correlations with VA.

Results—Twenty-nine eyes of 26 patients with RPD and 21 eyes of 16 age-matched AMD patients with drusen were included. Qualitatively, the choriocapillaris in areas with RPD showed focal dark regions without flow signal on OCTA (non-perfusion). The repeatability coefficient of PCAN was 0.49%. Eyes with RPD had significantly greater PCAN compared to eyes with drusen (7.31% and 3.88%, respectively; $P < 0.001$). We found a significant correlation between PCAN and VA for the entire dataset ($r = 0.394$, $P = 0.005$). When considering eyes with RPD separately, this correlation was stronger ($r = 0.474$, $P = 0.009$) but lost significance when considering eyes with drusen separately ($r = 0.175$, $P = 0.45$).

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B. Financial Disclosures: No financial disclosures.

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Conclusions—Eyes with RPD have significantly larger areas of choriocapillaris non-perfusion compared to eyes with drusen and no RPD. The correlation between PCAN and VA in this RPD population provides a potential mechanistic explanation for vision compromise in RPD compared to other forms of drusen in AMD.

Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among the elderly in the United States and Europe.^{1, 2} While drusen deposits in early AMD generally have mild impact on visual function, late stages of AMD, defined by choroidal neovascularization (CNV) or geographic atrophy (GA), are associated with significant visual impairment.³ Reticular pseudodrusen (RPD) represent a distinct AMD phenotype associated with worse visual function even at an early stage⁴⁻⁹, and an overall higher likelihood of progression to both forms of late AMD.¹⁰⁻¹⁶

In 1990, Mimoun and colleagues were the first to describe RPD as a peculiar yellowish pattern in the macula, more visible under blue light.¹⁷ In a histologic study, Arnold et al reported no evidence of drusen in one eye with RPD and speculated that the condition represented a loss of choroidal vessels and fibrous replacement of the choroidal stroma.¹⁰ Other studies, utilizing optical coherence tomography (OCT), have postulated that hyper-reflective material called subretinal drusenoid deposits (SDD), located not under (as typical drusen are) but above the retinal pigment epithelium (RPE), were responsible for the peculiar pattern of RPD.¹⁸ Since the original description, there has been significant controversy about the pathogenesis, composition and location of RPD.^{19, 20} Some studies suggest that SDD are not the cause of the peculiar pattern of RPD, and that SDD and RPD represent two distinct lesions.²¹⁻²³ Heiferman et al found significantly more RPD lesions on infrared reflectance (IR) than SDD lesions on *en face* OCT and the majority of SDD lesions were located greater than 30 μ m from the nearest RPD.²¹ Sohrab and colleagues used multimodal imaging, including IR, fundus autofluorescence (FAF), and *en face* OCT, and found that SDD were located adjacent to, but not overlying, RPD lesions.²² These findings were confirmed by Querques et al who showed that SDD lesions on OCT were located immediately adjacent to RPD lesions on indocyanine green angiography (ICGA).²³

Innovations in OCT technology, such as swept-source (SS)-OCT²⁴ and *en face* OCT imaging²⁵, have allowed better visualization and quantitative assessment of the choroid. Pathological changes of the choroid in eyes with RPD have been extensively documented using various OCT methods.²⁵⁻²⁸ A number of studies have reported significantly thinner choroid in AMD eyes with RPD compared to those without RPD^{6, 23, 29}, while other studies refuted the association of RPD with overall choroidal thinning.²⁸ These studies added further evidence to the lack of agreement on the pathology of RPD. Two studies using multimodal imaging, including *en face* OCT, suggested that RPD patterns, but not SDD, localize to the choroidal stroma and follow large choroidal vessels.^{22, 30} Querques et al provided further evidence of the close relation of RPD lesions and the choroidal stroma through the use of ICGA. In this study, they found that RPD patterns appeared as hypocyanescent lesions on ICGA that were closely abutting, but not overlying, the large choroidal vessels.²³ Furthermore, RPD are frequently found in monogenetic diseases such as Sorsby

fundus dystrophy³¹, Pseudoxanthoma elasticum³² and late onset retinal degeneration³³, conditions that are characterized by pathology at the level of Bruch's membrane and the RPE. While the pathogenesis of these entities is distinct, this association suggests that primary dysfunction of the choroid-Bruch's membrane-RPE complex may underlie the development of RPD in AMD.

Optical coherence tomography angiography (OCTA) is a non-invasive tool that provides depth-selective visualization of retinal and choroidal circulations. OCTA is based on motion detection, expressed as intensity of the decorrelation between two successive OCT scans. In addition to retinal vascular flow maps, OCTA enables visualization of choriocapillaris^{34, 35}, a highly specialized capillary layer (~10µm thick) located at the inner aspect of the choroid. One of the main functions of the choriocapillaris is to supply oxygen and metabolites to the RPE and photoreceptors.³⁶ Using OCTA, Alten and colleagues recently reported significantly reduced choriocapillaris vessel density and decorrelation signal index (a surrogate for choriocapillaris flow index) in RPD eyes compared to healthy control eyes, suggesting choriocapillaris functional compromise in RPD.³⁷

Based on the crucial role of the choriocapillaris for RPE and photoreceptor function and the significant visual function impairment in RPD, we initiated the current study to explore quantitative differences in the choriocapillaris, specifically focusing on eyes with RPD and comparing them to eyes with drusen in AMD. We hypothesized that eyes with RPD would have significantly greater areas of flow voids at the level of the choriocapillaris compared to eyes with other forms of drusen, and that the extent of choriocapillaris flow abnormality will correlate with visual acuity.

Methods

Patients were recruited for this cross-sectional study in the Department of Ophthalmology at Northwestern University in Chicago, Illinois between June 15, 2015 and May 9, 2016, and underwent a complete ophthalmological examination as part of their routine clinical work-up, including IR, FAF and SD-OCT with the Spectralis HRA+OCT (HRA; Heidelberg Engineering, Heidelberg, Germany). Subjects underwent fundus biomicroscopy and clinical examination by a single retinal physician (A.A.F.). This study was approved by the Institutional Review Board of Northwestern University, followed the tenets of the Declaration of Helsinki and was performed in accordance with the Health Insurance Portability and Accountability Act regulations. Written informed consent was obtained from all participants.

Study Sample

Inclusion in this study required a diagnosis of early AMD with or without RPD, based on clinical exam and multimodal imaging findings, including color fundus photographs, FAF, IR, and SD-OCT. Of these subjects, we only included eyes whose OCTA imaging was acceptable based on lack of significant movement or shadow artifacts in the area of interest and signal strength index score greater than 60. We compared the OCT angiogram to the *en face* structural OCT and cross-sectional OCT to identify shadow artifacts. If focal attenuation of the OCT signal was seen affecting all layers of the retina and choroid, we

graded this as a shadow artifact and this scan was not included in the study. Large dark lines on the *en face* angiogram were graded as motion artifacts and these scans were also omitted from the analysis. A total of 13 eyes were not included in the analysis due to motion and shadow artifacts (n=6) or low signal strength scores (n=7).

RPD were defined as a yellowish reticular pattern in the macula on clinical examination, which appear as hypo-reflective lesions interspersed against a background of mild hyper-reflectance on IR.^{10, 38} On FAF, RPD were defined as ill-defined areas of decreased FAF surrounded by areas of increased FAF signal.³⁹ IR and FAF imaging were used to confirm RPD in eyes suspected to have RPD based on clinical examination. Eyes were designated as early AMD if they had macular drusen, equivalent to grades 2 or 3 on the Age-Related Eye Disease Study (AREDS) scale.³⁹

Based on fundus biomicroscopy and multimodal imaging, we separated eyes into three groups: pure RPD (no associated drusen), RPD plus drusen (combination of RPD and regular drusen), and drusen (drusen without RPD). The pure RPD and RPD plus drusen groups were later combined in our analysis to form a single 'RPD' group based on the similarities of their qualitative and quantitative findings in the choriocapillaris on OCTA. These similarities included nearly identical choriocapillaris non-perfusion calculations and the appearance of "dark spots" in the choriocapillaris that were indistinguishable between these groups. None of the eyes in the drusen group, or their fellow eye, showed the peculiar reticular pattern in the macula on any imaging modality. Monocular, best-corrected visual acuity (BCVA) using existing correction was determined using Snellen eye charts under dim illumination for all patients.

Exclusion criteria included eyes diagnosed as having late AMD (AREDS grade 4), defined by the presence of CNV and/or central GA, eyes that had undergone intravitreal anti-vascular endothelial growth factor treatment, and eyes with other retinal disease or abnormalities, such as myopia greater than -2.00 diopters spherical equivalent, which may potentially contribute to choriocapillaris non-perfusion. In order to avoid optical artifacts that may potentially compromise OCTA image quality, we excluded eyes that had undergone surgical retinal repair and those with evidence of significant cataracts, graded above nuclear opalescence grade 3 or nuclear color grade 3.

Optical Coherence Tomography Angiography Imaging

To obtain angiograms of the choriocapillaris, we used the RTVue-XR Avanti OCTA system (Optovue Inc., Fremont, California) with split-spectrum amplitude-decorrelation angiography (SSADA) software.⁴⁰ This instrument has an A-scan rate of 70,000 scans per second and uses a light source centered on 840 nm and a bandwidth of 45 nm. Two consecutive B-scans (M-B frames), each containing 304 A-scans were captured at each sampling location and SSADA was used to extract OCTA information. We obtained 2×2 mm² scans (for highest possible image quality) in areas with RPD, using IR images as a guide. OCTA images were obtained in RPD areas primarily along the superior arcade, with occasional images nasal to the fovea and along the inferior arcade, guided by the location with maximum RPD. All scans used in the quantitative analyses were obtained in the outer sectors or outside the outer sectors of the Early Treatment Diabetic Retinopathy Study

(ETDRS) grid.⁴¹ For each eye, we obtained 1-4 individual OCTA scans, based on the extent of RPD and patient cooperation. OCTA images were obtained in similar locations in AMD eyes without RPD. One age-matched healthy eye was included in Figure 1 to show the appearance of normal choriocapillaris on this device. In addition to segmentation lines, the OCTA has the option of displaying flow (decorrelation) signal on the cross-sectional OCT, appearing as red flow pixels. The device also displays an *en face* structural OCT image corresponding to the layer of the angiogram being viewed. We utilized these functions to determine if the software-given *en face* angiogram of the choriocapillaris slab was indeed visualizing the choriocapillaris, from an anatomical standpoint (Figure 1). We also assessed the relation between the *en face* structural OCT and *en face* angiogram of the choriocapillaris.

Image Processing

The image-processing algorithm was designed to calculate the percent choriocapillaris area of non-perfusion (PCAN), defined as the percentage of pixels in the choriocapillaris below a “non-perfusion” global threshold. The equation for PCAN is the number of pixels that fall below the threshold divided by the total number of pixels in the image. In addition, we designed the algorithm to remove shadow artifacts that may confound the PCAN measurement. The flowchart of the algorithm is presented in Figure 2. Our algorithm relied on global thresholding of the choriocapillaris OCTA image in order to separate perfused choriocapillaris from non-perfused areas. Therefore, to identify the global threshold of “non-perfusion”, we first exported *en face* OCTA images of the avascular outer retina (slab with inner boundary located 70 μm below inner plexiform layer and outer boundary located 30 μm below the RPE reference). The images were then imported into ImageJ software (National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/index.html>). Finally, the global threshold was calculated as the mean of all the pixel values in the avascular outer retina image. All choriocapillaris pixels falling below this global threshold were considered “non-perfusion”.

We considered that projection and shadow artifacts from retinal vessels could potentially confound measurements of choriocapillaris non-perfusion. Superficial vessels left obvious shadows on the choriocapillaris (Figure 4). In addition, we also observed similar choriocapillaris shadow artifacts underneath drusen (Figure 3). We also considered the possibility that SDD could produce shadow artifacts, and thereby confound the measurements. To examine this question, we created a structural *en face* OCT map of SDD, using a ~5 microns thick slab segmented at the inner segment-outer segment junction. This slab clearly highlighted SDD as hyper-reflective lesions based on the location of SDD above the RPE. However, by using point-to-point correlation, we found that SDD’s identified on OCT B-scans and structural OCT slabs showed no shadow artifacts (Figure 3). Two graders (A.A.F. and P.L.N.) confirmed these observations. Therefore, in the final algorithm we only addressed drusen and superficial retinal blood vessels as potential confounders for PCAN.

To address these potential confounders, our algorithm identified the overlying drusen and retinal blood vessels, and removed these areas from the PCAN calculations (Figure 2, red). In order to identify shadow artifacts generated by drusen, we created a map of the drusen

using an *en face* structural OCT slab (~28 microns thick) at the level of the RPE. We used this slab because it highlighted drusen elevating the RPE as hyporeflective lesions. In order to identify shadows from retinal blood vessels, the superficial capillary angiogram was also segmented. To perform the PCAN calculation, the drusen image, superficial capillary plexus angiogram, and the choriocapillaris angiogram were imported into a custom MATLAB software (MathWorks, Inc., Natick, Massachusetts). The software automatically calculated the percentage of choriocapillaris pixels below the global “non-perfusion” threshold, and excluded from this measurement areas of choriocapillaris underlying drusen and superficial retinal vessels. In addition, the algorithm removed areas with OCTA segmentation errors. If multiple scans were included for an eye, the average PCAN was taken to represent the eye. Figure 4 shows an example image from an eye with both drusen and RPD. Our algorithm identified the drusen and superficial retinal vessels, and subsequently removed these areas from the final PCAN calculation.

Statistical Methods

Statistical analysis was performed using the SPSS software (version 17.0; SPSS, Inc., Chicago, IL). We ran independent samples t-tests to for all demographic information (Table). We performed independent samples t-tests to compare the mean PCAN between the groups.

The BCVA for each eye was converted to the logarithm of the minimum angle of resolution (logMAR) as previously described.⁴² Shapiro-Wilk tests for logMAR visual acuity were statistically significant, indicating this data was not normally distributed. Therefore, we performed three separate Spearman rank correlations to examine the relationship between PCAN and the logMAR visual acuity for (1) all eyes in the study, (2) eyes with pure RPD combined with eyes with RPD plus drusen, and (3) eyes with drusen (without RPD). We also used the Spearman rank test to examine whether age was correlated to logMAR visual acuity or PCAN. We defined $P < 0.05$ as statistically significant for all tests.

Repeatability of OCTA and Measurements

To test the repeatability of the OCTA and PCAN measurements, we obtained two consecutive scans of the same area of the retina in 26 eyes (7 RPD, 11 drusen, and 8 healthy eyes). After the first scan, we asked the patient to sit back and then return to the instrument, where we refocused and captured the second image. We followed the same protocol as above to calculate PCAN for the two images from each eye. We then plotted the difference against the mean for each of the repeated measures (Bland-Altman plot) to ensure that the following statistical tests for repeatability were appropriate. We then calculated the within-subject standard deviation and the coefficient of repeatability for PCAN/OCTA using previously described methods.⁴³

Results

A total of 29 eyes of 26 consecutive patients with RPD (19 female [73.1%]; mean age, 80.2 ± 7.1 years; range, 65-91 years) and 21 eyes of 16 consecutive AMD subjects with drusen (9 female [56.3%]; mean age, 79.0 ± 4.9 years; range, 72-88 years) were included in the analysis. The overall demographic and clinical characteristics of the two groups were

matched (Table). Mean BCVA for eyes with RPD was 0.17 ± 0.19 logMAR (range, 0.0-0.5) and for AMD eyes with drusen was 0.09 ± 0.13 logMAR (range, 0.0-0.48).

Qualitative Analysis of the Choriocapillaris

We confirmed that the built-in software segmentation of the choriocapillaris was indeed visualizing the anatomical choriocapillaris based on the assessment of the segmentation lines and red flow signal on cross-sectional OCTA. In healthy eyes, the choriocapillaris showed a diffuse flow signal, immediately below Bruch's membrane (Figure 1). *En face* structural OCT of the choriocapillaris did not reveal any additional insights or correlation with the flow signal of the choriocapillaris angiogram and was therefore not analyzed further in this study (Figure 1).

The choriocapillaris in RPD eyes had an overall "darker appearance" with numerous, focal "dark spots" lacking flow signal in areas with RPD (Figure 5, Second row). These dark spots were qualitatively larger in size compared to the spots of non-flow seen in AMD eyes with drusen. Within an individual RPD eye, these spots were characteristically more pronounced in areas with visible RPD on IR images, compared to locations without RPD, with a relatively distinct transition (Figure 5, Top and Second row). In contrast, AMD eyes with drusen showed a more uniform choriocapillaris flow signal on OCTA without regional differences (Figure 5, Third row).

Quantitative Analysis of the Choriocapillaris

An average of 2.1 scans per eye (each scan: 2×2 mm²) were used to calculate PCAN (choriocapillaris non-perfusion). We found no statistically significant difference in PCAN between the "pure RPD" and "RPD plus drusen" groups ($7.30\% \pm 1.37\%$ and $7.31\% \pm 1.41\%$, respectively; $P = 0.99$). We therefore combined these groups into one 'RPD' group. PCAN was significantly greater in eyes with RPD compared to AMD eyes with drusen ($7.31\% \pm 1.37\%$ for RPD and $3.88\% \pm 1.07\%$ for drusen; $P < 0.001$) (Figure 6).

Using Spearman rank correlations, we found a statistically significant correlation between PCAN and logMAR visual acuity for the entire dataset ($r = 0.394$, $P = 0.005$). When considering the combined group of eyes with pure RPD and RPD plus drusen, this correlation was stronger ($r = 0.474$, $P = 0.009$) (Figure 7). Interestingly, when eyes with drusen (without RPD) were considered separately, the correlation lost significance ($r = 0.175$, $P = 0.45$). We found that age did not have a potential confounding effect on choriocapillaris PCAN ($r = 0.169$, $P = 0.24$), or logMAR visual acuity ($r = 0.212$, $P = 0.14$).

Repeatability of Measurements

The Bland-Altman plot revealed no skewing of the relationship between the difference and the mean of the PCAN calculations of 26 eyes (7 RPD, 11 drusen and 8 healthy eyes) with repeated measurements (Figure 8). This allowed us to calculate the repeatability coefficient without mathematical transformations. The mean of the differences was $-0.06 \pm 0.25\%$ for repeated OCTA PCAN calculations. The within-subject standard deviation and repeatability coefficient of PCAN for these 26 eyes were 0.18% and 0.49%, respectively.

Discussion

Using an OCTA metric for choriocapillaris non-perfusion, we found that RPD eyes had significantly larger non-perfused area compared to eyes with drusen without RPD. This finding, along with previous reports of choroidal changes in eyes with RPD^{22, 23, 25-30}, suggests a significant and potentially primary role for the choroid in the pathogenesis of RPD. We also found the percentage of non-perfused choriocapillaris on OCTA correlated to visual acuity in the entire dataset of AMD eyes. This correlation was stronger when RPD eyes were considered separately. This finding likely reflects the important role of the choriocapillaris for photoreceptor/RPE metabolic function, which may be significantly altered in RPD eyes. The coefficient of repeatability for the OCTA and our choriocapillaris non-perfusion measurement (PCAN) was 0.49%, confirming that the difference between eyes with RPD and eyes with drusen (without RPD) can be attributed to real differences in choriocapillaris perfusion rather than inter-scan variability.

There are several potential hypotheses that can be put forth to explain the presence of non-perfusion of the choriocapillaris on OCTA. These include reduced choriocapillaris flow velocity below the decorrelation threshold (less than 0.3mm/sec), a reduced number of choriocapillary vessels per unit area, or decreased choriocapillaris vessel caliber. Another possibility could be that the choriocapillaris is completely obliterated in areas with RPD allowing us to visualize the flow in the underlying Sattler's layer of medium sized choroidal vessels (manuscript in review).⁴⁴ All of these scenarios would likely result in hypoxia of the RPE and photoreceptors, and a reduction in the metabolic supply necessary for photoreceptor visual function. We hypothesize that this decrease in choriocapillaris perfusion is global in eyes with RPD explaining the correlation between visual acuity and non-perfusion of the choriocapillaris in these eyes. We sought to image areas with the highest concentration of RPD and avoid areas likely to have drusen, such as the central macula, since they cause extensive shadow artifacts in the choriocapillaris (Figures 2-4).

Histological studies of the choroid in eyes with a clinical pre-mortem diagnosis of RPD are few in number. In a histology study of one eye, Sarks and colleagues reported a patchy loss of choriocapillaris and a thin choroid in the area of RPD.⁴⁵ Arnold et al reported a significant loss of choroidal vessels of the Sattler layer and increased spacing between large choroidal veins, suggesting that fibrosis of the choroidal stroma and loss of vascularity were responsible for the development of RPD.¹⁰ In a more recent histologic study, Greferath and colleagues reported SDD in the outer nuclear layer juxtaposed to photoreceptor outer segments, along with overlying photoreceptor disruption and loss.⁴⁶ Although Greferath reported a lack of choroidal changes in association with RPD, this study focused on cross-sections of larger vessels in the choroid and provided no quantitative flatmount studies or detailed endothelial immunohistochemical or electron microscopic methods that would be necessary to evaluate the integrity of the choriocapillaris.

Studies utilizing OCT have reported an overall thinner choroid in eyes with RPD compared to AMD eyes without RPD^{23, 29}, a finding that was refuted by others.²⁸ These differences may be explained by the regional distribution of choroidal changes in RPD. In fact, although Querques et al found an overall thinner choroid in RPD eyes, they also found that the

superior macula was significantly thicker than any other location of the retina.²³ These findings also correlate with the regional changes we have seen in qualitative OCTA. Larger zones of choriocapillaris non-flow were located in regions of the choroid that underlie the location of RPD in our images, with distinct transition to zones of relatively normal choriocapillaris outside the RPD zone (Figure 5). Furthermore, if fibrotic replacement of medium sized choroidal vessels is present in RPD as suggested by Arnold,¹⁰ this would potentially confound choroidal thickness measurements on structural OCT. Our study revealed a significant reduction in choriocapillaris perfusion in eyes with RPD compared to AMD eyes with drusen without RPD. These studies, in aggregate, point to an important role of the choroid, specifically the choriocapillaris, in the pathogenesis of RPD, which deserves further study.

While our understanding of the newly developed technology of OCTA is in its nascent stages, the application of this technology shows great promise as a powerful new approach to investigate the choriocapillaris in AMD. Alten and colleagues recently compared the choriocapillaris in eyes with RPD to age-matched healthy controls using two OCTA parameters, vessel density and decorrelation index. These authors found that both parameters were significantly reduced in RPD eyes.³⁷ Interestingly, they also found that reduced choriocapillaris vessel density correlated to the number of RPD lesions, which seems to fit with our qualitative assessment of larger dark spots in areas of RPD. For their analysis, Alten and colleagues used $6 \times 6 \text{ mm}^2$ scans, which significantly reduce the quality of the OCTA image ($\sim 19.7 \text{ } \mu\text{m}$ between B-scans), compared to $2 \times 2 \text{ mm}^2$ scans used in the current study ($\sim 6.6 \text{ } \mu\text{m}$ between B-scans). Furthermore, their study did not take into account the potential shadowing artifact from overlying retinal vessels. Our study extends their findings by showing that eyes with RPD had significantly more choriocapillaris non-perfusion than eyes with drusen without RPD, using higher quality $2 \times 2 \text{ mm}^2$ images and excluding shadow artifacts. Furthermore, we demonstrate a functional dimension to these imaging findings as we show a significant correlation between choriocapillaris non-perfusion and visual acuity in RPD eyes.

The visual functional defects in eyes with RPD are generally believed to affect the rod more than cone functions. Flamendorf and colleagues found a significant increase in time required for dark adaptation, a measure of rod function, in eyes with RPD compared to eyes with AMD without RPD.⁴ Hogg et al similarly found that scores on the Smith-Kettlewell low-luminance acuity test were significantly lower among AMD eyes with RPD compared to those without these lesions.⁶ Other studies focused on cone and rod mixed function with microperimetry and found a significant reduction in retinal sensitivity in eyes with RPD compared to early AMD eyes without RPD.^{5, 7, 8} Future studies comparing the extent of choriocapillaris non-perfusion to dark adaptation latency or microperimetry metrics would be important to further confirm our hypothesis that choriocapillaris flow abnormalities underlie the reduced visual function in eyes with RPD.

In this study, we excluded eyes with advanced AMD pathology, as both GA and CNV could have profound effects on the choriocapillaris, and our goal was to compare the choriocapillaris changes in eyes with RPD to those with early AMD and drusen without RPD. That being said, based on our choroidal findings, the increased risk of late AMD in

eyes with RPD warrants some discussion. Population studies have found that RPD are associated with a higher incidence of both forms of late AMD, GA and CNV.^{16, 47} While the exact mechanisms of progression to late AMD remain unknown, especially in RPD, current and previous studies point to the choriocapillaris and choroid as important pieces to this puzzle. Using donor eyes, McLeod and colleagues found that loss of the choriocapillaris occurs underneath the healthy RPE in a circumferential zone outside the location of CNV in eyes with this form advanced AMD, and proposed that primary choriocapillaris loss with secondary angiogenic signaling from the hypoxic RPE may result in the proliferation and differentiation of endothelial cells, ultimately leading to CNV.⁴⁸ Biesemeier and colleagues similarly found large areas of choriocapillaris loss around CNV and suggested that initial choriocapillaris breakdown precedes RPE and retinal degeneration in neovascular AMD, providing evidence to suggest that AMD is a vascular disease.⁴⁹ Data from our study shows that eyes of RPD have greater flow voids in the choriocapillaris. If choriocapillaris atrophy is the hypoxic precursor triggering the onset of CNV, this could potentially explain the higher risk of progression to CNV in eyes with RPD.

The significant choriocapillaris compromise in RPD eyes lends further support to the hypothesis that SDD accumulation on the apical aspect of the RPE may be a non-specific reaction of the RPE to hypoxia. This has also been shown recently by Kurihara and colleagues using transgenic mice with RPE hypoxia-induced metabolic stress (conditional *vh1*/knockout in the RPE).⁵⁰ Within 14 days of hypoxic induction, these mice developed deranged RPE metabolism leading to accumulation of lipid deposits in Bruch's membrane, as well as within the RPE and extending into the subretinal space, simulating the appearance of AMD and SDD. Further follow up showed photoreceptor degeneration and loss of visual function in this model. We therefore extrapolate from these studies and our findings to suggest that RPE hypoxia (in the setting of choroidal capillary non-perfusion) is the primary insult in RPD, which precedes the development of SDD. These findings are further supported by the OCT and ICGA findings of juxtaposition of SDD and abnormal choriocapillaris.^{22, 25} We hypothesize that the life cycle of RPD begins with choriocapillaris closure, which appears on IR as hyporeflective lesions and on OCTA as flow voids. This leads to secondary RPE hypoxia, which in turn leads to is deranged metabolism within the RPE, the accumulation of SDD on OCT, and ultimately photoreceptor degeneration with disappearance of the SDD.²¹

Limitations of this study include a relatively small number of eyes. Larger cohort studies are needed to confirm our findings. Excluding eyes with late AMD greatly limited the number of eyes we were able to include in the analysis, but also reduced the number of confounding variables. Not including eyes with significant motion artifact, shadow artifact and low signal strength also reduced our sample size, but allowed us to make more definitive conclusions from our results. OCTA imaging of the choriocapillaris is relatively recent development³⁷ and must be validated using larger cohorts of patients, other OCTA systems, and confirmed with histological studies. Furthermore, hyper-reflective retinal layers, such as the RPE, greatly attenuate the signal strength of the underlying layers with our current SD-OCTA device. Future studies using swept-source OCTA, which uses a longer wavelength allowing for better penetration of the RPE, would be desirable. Finally, we believe that

psychophysical tests such as dark adaptation will be important to study rod photoreceptor function in these eyes, but these were beyond the scope of the current study.

In conclusion, we demonstrate a novel OCTA metric with high repeatability for measuring choriocapillaris non-perfusion, PCAN. Using PCAN, we found significantly reduced flow in eyes with RPD compared to age-matched early AMD eyes with drusen (without RPD). This finding correlates well with histological findings in eyes with RPD^{16, 47}, as well as our qualitative assessment of the choriocapillaris on OCTA in areas of RPD. Furthermore, decreased choriocapillaris flow showed a significant correlation with visual acuity in eyes with RPD, compared to AMD eyes with drusen. Our findings emphasize the importance of the choriocapillaris to visual function in eyes with RPD, and suggest the need for future studies that explore relationships between choriocapillaris flow and more sensitive psychophysical measures of vision.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

A. Funding/Support: This work was funded in part by NIH DP3DK108248 (AAF), NIH F30EY026472 (BTS) and research instrument support by Optovue, Inc., Fremont, California, USA. The funders had no role in study design, data collection and analysis, data interpretation, decision to publish, or preparation of the manuscript.

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Figure 1. Optical Coherence Tomography Angiography (OCTA) Imaging of the Choriocapillaris
 Example *en face* OCTA images of the choriocapillaris (left) and *en face* structural OCT (right), in a healthy age-matched eye (Top) early age-related macular degeneration with drusen (Middle), and reticular pseudodrusen (RPD; Bottom). OCT B-scans with red flow overlay are shown below each OCTA image highlighting the relatively uniform flow signal immediately below Bruch's membrane, with relevant segmentation boundaries (red lines) confirming the angiogram is of the choriocapillaris. OCT B-scans without red flow signal below each structural OCT image show the structural components of the choriocapillaris, which do not consistently correlate with flow signal of the choriocapillaris angiogram.

Figure 2. Algorithm for Artifact Removal and Calculation of Percent Choriocapillaris Area of Non-Perfusion (PCAN)

Optical coherence tomography (OCT) and OCT angiography (OCTA) images covering a $2 \times 2 \text{ mm}^2$ area were acquired by the Angiovue device. Retina layer segmentation was performed on the three-dimensional (3-D) structural OCT image to obtain the locations of the retinal layers. The 3-D OCT image was converted into a two-dimensional retinal pigment epithelium (RPE) structure image by semi-automatically selecting the RPE layer and obtaining the *en face* slab ($\sim 28 \mu\text{m}$ thick) to highlight drusen and retinal vessels that may cast shadows on the choriocapillaris. The 3-D OCTA image was converted into two *en face* angiograms: one for the superficial retinal capillary vasculature and one for the choriocapillaris. The segmentation for the superficial angiogram extended from $3 \mu\text{m}$ below the inner limiting membrane to $15 \mu\text{m}$ below the inner plexiform layer, while the segmentation for the choriocapillaris was taken from the bottom of the RPE to $\sim 28 \mu\text{m}$ below. The segmentation lines are shown on the OCT B-scan below each image. To obtain masks of the shadow artifacts, the RPE structure (drusen mask) and superficial angiogram were globally thresholded. To obtain a mask of the choriocapillaris non-perfusion, the choriocapillaris angiogram was first inverted and then thresholded based on the outer retinal slab; all choriocapillaris pixels with intensity below the average pixel intensity of the outer retina (noise-level) were considered non-perfusion in the choriocapillaris. The artifact masks were removed from the choriocapillaris non-perfusion mask, to avoid false counting of shadow artifacts as non-perfusion. Finally, the PCAN in the choriocapillaris non-perfusion

mask is calculated by dividing the area below the threshold by the remaining area of choriocapillaris.

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Figure 3. Subretinal Drusenoid Deposits (SDD) and Drusen on Optical Coherence Tomography Angiography (OCTA)

Enlarged view from choriocapillaris angiograms in eyes with reticular pseudodrusen (RPD) centered on the location of SDD (Top row). Enlarged view of OCT B-scan from the OCTA device centered on SDD of varying size and shape (Second row). Regardless of the size of the SDD lesion, they did not produce shadowing artifacts on the choriocapillaris. Enlarged view from choriocapillaris angiograms in eyes with drusen centered on the location of the drusen (Third row). Enlarged view of OCT B-scan from the OCTA device centered on drusen of varying size and shape (Bottom row). Drusen produced shadows on the choriocapillaris that may be artifactually counted as non-perfusion.

Figure 4. Elimination of Shadow Artifacts on the Choriocapillaris of an Eye with Both Drusen and Reticular Pseudodrusen (RPD)

Top left: Color fundus photograph (CFP) with location of optical coherence tomography angiography (OCTA) image (green box). Bottom left: Infrared reflectance image with visible hypo-reflective RPD lesions. Top middle-left: OCTA image ($2 \times 2 \text{ mm}^2$) of the superficial capillary plexus (SCP) with B-scan below showing red and green segmentation boundary lines. Bottom middle-left: SCP with overlaid red pixel mask of the brightest pixels, which will be eliminated from choriocapillaris non-perfusion calculation. Top middle-right: Original OCTA image of the choriocapillaris with B-scan below showing red segmentation boundary lines. Bottom middle: OCTA of the choriocapillaris with green pixel mask representing pixels falling below the pixel intensity threshold. Top right: *En face* structural OCT image segmented at the retinal pigment epithelium (RPE) with B-scan below showing red segmentation boundary lines. Dotted red line shows location of B-scans. Drusen and large retinal vessels appear dark. Bottom middle-right: *En face* structural OCT with red pixel mask (darkest pixels in this *en face* image) representing drusen and retinal vessels, areas which will be eliminated from the choriocapillaris non-perfusion calculation. The diffuse red pixels in the top of the image represent an area where the segmentation boundaries failed to follow the contour of RPE, which will also be eliminated from the calculation. Bottom right: OCTA of choriocapillaris with the non-perfusion (green) pixel and artifact (red) pixel masks. Red pixels were eliminated from the image before the final calculation of the “percent choriocapillaris area of non-perfusion” (PCAN) from the remaining image.

Figure 5. Distinct Appearance of the Choriocapillaris Underlying Reticular Pseudodrusen (RPD)

Top row: Infrared reflectance (IR) images of three eyes with RPD and corresponding optical coherence tomography angiography (OCTA) *en face* images directly below. The red lines indicate the transition zone from RPD present (superiorly) to RPD absent (inferiorly).

Second row: The OCTA images of the choriocapillaris in RPD reveal a distinct pattern of numerous, focal “dark spots” lacking any flow signal underlying the zones of RPD as seen on IR. RPD is present above the red line in the OCTA scans. Third row: OCTA of the choriocapillaris in three eyes with early age-related macular degeneration (AMD) with drusen without RPD. OCTA shows a more uniform flow signal throughout the scan without the obvious appearance of the “dark spots,” which are seen in eyes with RPD. Bottom row:

The IR images of the three AMD eyes with drusen without RPD, corresponding to OCTA seen in the third row, confirm absent RPD patterns and absent regional choriocapillaris flow changes.

Figure 6. Choriocapillaris Non-Perfusion on Optical Coherence Tomography Angiography (OCTA) is Significantly Greater in Eyes with Reticular Pseudodrusen (RPD)

The average “percent choriocapillaris area of non-perfusion” (PCAN) was 7.31% for eyes with RPD and 3.88% for eyes with early age-related macular degeneration with drusen without RPD ($P < 0.001$). Scale bars represent standard deviations (1.37% and 1.07% for RPD and drusen, respectively).

Figure 7. Choriocapillaris Non-Perfusion on Optical Coherence Tomography Angiography (OCTA) Correlated Significantly with Visual Acuity

Left: Spearman rank correlation between “percent choriocapillaris area of non-perfusion” (PCAN) and logarithm of the minimum angle of resolution (logMAR) visual acuity for all eyes with age-related macular degeneration (AMD) included in the study ($r = 0.394$, $P = 0.005$). This included eyes with drusen and eyes with reticular pseudodrusen (RPD). Right: The correlation between PCAN and logMAR visual acuity was stronger when only eyes with RPD and eyes with RPD plus drusen were considered ($r = 0.474$, $P = 0.009$).

Figure 8. Repeatability of Optical Coherence Tomography Angiography (OCTA) and Image Processing

The Bland-Altman plot comparing two repeated measurements of the same location on retina with the OCTA device, and the “percent choriocapillaris area of non-perfusion” (PCAN) for each scan. The difference in PCAN scores between the two repeated measurements are plotted against their mean to explore the relation between the difference and the mean. No trend was apparent, allowing us to calculate the coefficient of the repeatability without mathematical transformations. The mean of the differences was -0.06 (middle dotted line) and standard deviation (SD) of the differences was 0.25 ($\text{Mean} \pm 1.96 \times \text{SD}$: top and bottom dotted lines).

Table**CHARACTERISTICS OF STUDY PARTICIPANTS**

	<i>RPD Group</i>	<i>AMD Non-RPD Group</i>	<i>P-value</i>
No. Subjects (No. eyes)	26 (29)	16 (21)	
No. Female (%)	19 (73.1)	9 (56.3)	
Mean Age (y) $\pm$ SD	80.2 $\pm$ 7.1	79.0 $\pm$ 4.9	0.56
Mean BCVA (logMAR) $\pm$ SD	0.17 $\pm$ 0.19	0.09 $\pm$ 0.13	0.10
Mean Refractive Error (D) $\pm$ SD	-0.23 $\pm$ 1.05	-0.51 $\pm$ 1.07	0.36

The two study groups have a similar mean age, visual acuity and refractive error. Abbreviations: AMD = Age-related macular degeneration, BCVA = Best corrected visual acuity converted to logarithm of the minimum angle of resolution (logMAR), D = Diopter, P-value = Significance value from two-tailed independent samples *t* test, RPD = Reticular pseudodrusen, SD = Standard deviation, Y = years.