Localized Reticular Pseudodrusen and Their Topographic Relation to Choroidal Watershed Zones and Changes in Choroidal Volumes

Florian Alten, Christoph R. Clemens, Peter Heiduschka, and Nicole Eter

Department of Ophthalmology, University of Muenster Medical Center, Muenster, Germany

Purpose. We identified a topographic relation of localized reticular pseudodrusen (RPD) to choroidal watershed zones (CWZ) and to changes in choroidal volumes (CV).

Methods. We included 30 eyes of 30 patients with RPD in an area <10 mm² and no other retinal alteration (76.7 ± 6.9 years). Patients underwent spectral-domain optical coherence tomography (SD-OCT), enhanced depth imaging (EDI OCT), fluorescein video-angiography (vFA), indocyanine green video-angiography (vICG), and confocal scanning laser ophthalmoscopy (cSLO). vICG was evaluated for the presence, localization, and configuration of CWZ. Retinal areas affected by RPD were measured, and their localization was determined in relation to CWZ. CV was measured using a choroidal thickness map of the posterior pole and the Early Treatment of Diabetic Retinopathy Study (ETDRS) grid.

Results. In all study eyes, RPD could be demonstrated clearly in SD-OCT, EDI-OCT, FA, ICG, and cSLO. CWZ were identified in 25 eyes (83.3%). The area affected by RPD was 7.45 ± 2.25 mm². RPD area was located fully or partly within the CWZ in 22 eyes (88.0%). Mean CV in the full ETDRS grid area was reduced significantly (4.49 ± 1.44 mm³). Foveal CV and CV in the grid sector affected mostly by RPD were significantly diminished to 0.14 ± 0.05 mm³ and 0.85 ± 0.38 mm³, respectively.

Conclusions. The site of localized RPD seems to be related to presence and site of CWZ, suggesting that choroidal hypoxia may have a role in RPD pathogenesis. Reduced CV in eyes with localized RPD could be demonstrated and may be cause or consequence of RPD development.

Keywords: age-related macular degeneration, reticular drusen, reticular pseudodrusen, subretinal drusenoid deposits, scanning laser ophthalmoscopy, indocyanine green angiography, spectral domain optical coherence tomography, enhanced depth imaging optical coherence tomography, choroidal watershed zone.

Different studies were able to demonstrate a strong relationship between reticular pseudodrusen (RPD) and age-related macular degeneration (AMD), and its progression.1-6 However, the pathophysiologic biogenesis of RPD still is unclear. Various theories have been proposed in the literature. Rudolf et al. hypothesized that RPD are metabolism products that may be released from the apical as well as the basal surface of the RPE cell due to a deranged polarity.7 Sarks et al. proposed that undigested disorganized photoreceptor outer segments represent this distinct finding.8 Sohrab et al. recently provided results suggesting that the arrangement and pattern of RPD are related closely to the choroidal stroma and the choroidal vasculature.9 A recent study by Querques et al. revealed an overall thinned choroid in patients with RPD, and a choroidal atrophy and fibrosis underlying RPD, favoring the thesis that the choroid may represent the point of origin of RPD pathogenesis.10 Although major progress has been made recently in identifying inflammation mechanisms, as well as certain risk genotypes to have a key role in AMD pathogenesis,11,12 there still is strong evidence that choroidal ischemia also represents a contributing factor in the development of AMD. Hemodynamic studies using color Doppler imaging, flowmetry, fluorescein angiography, and indocyanine green angiography disclosed reduced macular choroidal perfusion in eyes with AMD, suggesting that impaired choroidal circulation may promote the pathogenesis of dry and exudative AMD.13-22 Associations between a reduced choroidal perfusion and different phenotypes of AMD have been reported for choroidal neovascularization (CNV),14,15,19 conventional drusen,13,16,17 geographic atrophy (GA),13,16 disease severity,18,20 disease risk factors,21 and disease progression.22 Taken together, these findings support the theory that a vascular ischemic component is involved in the multifactorial pathomechanism of AMD.

In a number of preliminary in vivo experimental studies on the choroidal vascular bed, Hayreh showed that posterior ciliary arteries and choriocapillaris are a segmentally structured end-arterial system.23 Each choriocapillaris lobe represents an individual unit without functional Anastomosis with adjacent lobules. Consequently, the choroid is an end-arterial tissue identical to, for example, kidney or cerebrum. watershed zones are located at the borderline of perfusion areas of any two or more anatomic end-arteries and represent areas of relatively poor blood flow supply, and, thus, are most vulnerable to ischemia and hypoxia.24 The submacular choroid is an area...
where multiple choroidal watershed zones (CWZ) meet, which predisposes the macula to ischemia more than any other part of the posterior choroid. Whether choroidal ischemia, reduced choroidal blood flow, or any changes in choroidal architecture may be responsible for the development of RPD has remained unanswered to date.

The purpose of our study was to investigate in vivo a potential topographic relation of an area of localized RPD to CWZ, as well as to changes in choroidal thickness (CT) and choroidal volume (CV) in eyes showing exclusively RPD at an early stage. In contrast to previous studies on RPD, we tried to study this phenotype “at the beginning” of its development strictly including only subjects showing RPD restricted to a small area of the posterior pole.

METHODS

Population

Participants were recruited from the medical retinal clinic of the Department of Ophthalmology at University of Muenster Medical Center. For inclusion, patients had to present with distinct RPD in combined simultaneous confocal scanning laser ophthalmoscopy (cSLO) and spectral domain optical coherence tomography (SD-OCT) imaging of the posterior pole in at least one eye. To investigate a potential association between presence and site of CWZ, and the site of localized RPD phenotype as exactly as possible, we included only patients who showed localized RPD in a macular area smaller than 10 mm² and excluded those with RPD diffusely scattered over the posterior pole.

Study eyes were not eligible if any signs of conventional soft or hard drusen, CNV, GA, or pigment epithelium detachment due to AMD were observed in fundoscopy, SD-OCT, cSLO fundus autofluorescence (FAF), cSLO infrared reflectance (IR), or fluorescein angiography (FA). Intravitreal anti-VEGF therapy and vitreoretinal surgery in the medical history, as well as other vascular or inflammatory retinal pathologies also led to exclusion. Furthermore, eyes with dense lens opacities, corneal opacities, refractive surgery, or a history of intraocular inflammation also were excluded. Further exclusion criteria were refractive error > 2 diopters (D) due to the described relation of myopia and choroidal thinning. Best corrected visual acuity (BCVA) better than 0.5 of the study eye was required. Current medication and medical history were reviewed for the presence of chronic vascular diseases, since vascular diseases were reported to be associated with presence of CWZ and reduced foveal choroidal blood flow. Hypertension was defined as elevated arterial blood pressure, which generally was controlled by at least two different medications. Peripheral vascular disease was defined as having a history of lower extremity claudication. Diabetes mellitus was defined as having a history of multiple episodes of elevated blood glucose levels controlled by diet or medication. Coronary artery disease was defined as a history of angina pectoris, myocardial infarction, or coronary artery intervention. If one criterion was positive the patient was considered as having a chronic vascular disease.

We included 30 eyes of 30 patients with RPD in the posterior pole (22 females, 8 males, age 76.7 ± 6.9 years). Control patients were referred to the department for exclusion of retinal pathology in one eye. The healthy fellow eyes served as control eyes. They underwent the same examination protocol as eyes of the RPD group and were age-matched to RPD eyes. Refractive error > 2 D also led to exclusion in the control eyes. We included in the control group 30 healthy eyes of 30 control subjects (14 females, 16 males, age 75.4 ± 6.2 years) without RPD or other characteristics associated with AMD or any other retinal pathology. Informed consent from each subject for additional imaging beyond routine clinical examination and use of retinal imaging data for research was obtained. Research adhered to the tenets of the Declaration of Helsinki.

Imaging

All patients underwent fundus photography (Zeiss FF450; Carl Zeiss Meditec, Berlin, Germany), SD-OCT, enhanced depth imaging OCT (EDI-OCT), fluorescein video-angiography (vFA), indocyanine green video-angiography (vICG), and cSLO FAF and IR imaging (Spectralis; Heidelberg Engineering, Heidelberg, Germany). Using automated eye tracking and image alignment based on cSLO images, the software allows averaging a variable number of single images in real time (Automatic Real Time [ART] Module; Heidelberg Engineering). Images were viewed using Zeiss Visupac 4.2 and Heidelberg Eye Explorer 1.7.1.0.

Video-Angiography

vICG allows for clearly displaying the choroidal circulation, and for identification and delineating of CWZ. CWZ was defined as characteristic vertical, angled, or stellate-shaped zones of transient early-phase vICG hypofluorescence produced by the delayed filling of choriocapillaris (Fig. 1A). The delayed perfusion of the CWZ from the adjacent choriocapillaris gradually results in an evenly distributed background fluorescence. vICG was performed using 5 mg ICG dye (ICG-Pulsion; Pulsion Medical Systems, Feldkirchen, Germany) diluted in 5 mL aqueous solvent, injected into a peripheral vein in the arm. The field of view was set at 55°, and centered between optic disc and fovea. vICG was recorded during the first 45 seconds after injection showing the choroidal vascular system filling with dye. Excitation wavelength was at 787 nm and the range of transmitted light through the barrier filter was above 800 nm averaging 8 single frames in real time (ART Module; Heidelberg Engineering). Filling time was defined as the interval from dye injection until first detection of fundus fluorescence. After vICG, central images of middle and late phase ICG additionally were recorded. We evaluated vICG of patients with RPD and healthy age-matched control subjects for the presence and configuration of CWZ. Grading was performed independently by two masked observers. If the two observers disagreed, a third one was asked to arbitrate. Observers were instructed to use, apart from vICG, also corresponding vFA to identify the major arterial and venous circle and demarcation of CWZ (Fig. 1C). vFA was performed in the same manner using an intravenous (IV) injection of 5 mL fluorescein (Fluorescein Alcon; Alcon Pharma GmbH, Freiburg/Breisgau, Germany). Excitation wavelength was at 488 nm and the range of transmitted light through the barrier filter was 500 to 700 nm averaging 8 single frames in real time (ART Module; Heidelberg Engineering). After vFA, central images of middle and late phase FA were recorded to rule out any retinal pathology.

Confocal Scanning Laser Ophthalmology

Using the integrated measurement tool of the Heidelberg Eye Explorer, we outlined the area affected by RPD and included only patients if the area was smaller than 10 mm². Measurements were performed independently by two masked readers and values subsequently were averaged. This way, we wanted to exclude patients with a progressed RPD pattern over the
whole posterior pole and warrant that we analyze patients at the beginning of the localized RPD phenotype.

cSLO imaging (FAF, excitation λ = 488 nm, range of transmitted light through barrier filter 500–700 nm, IR λ = 830 nm, Spectralis; Heidelberg Engineering) was performed with a minimum resolution of 768 × 768 pixels. The field of view was set at 30° × 30° and centered on the macula, optic nerve head, and temporal macula. A retinal FAF and IR montage image was calculated automatically for each eye using the compute composite mode of the Heidelberg Eye Explorer that merges the three captured retinal fields (Fig. 1B).

Spectral Domain-Optical Coherence Tomography

SD-OCT images were obtained by using 25° × 30° volume scans consisting of 61 equally spaced horizontal averaged B-scans. The field of view was centered on the fovea. Scans were saved for analysis after 50 frames were averaged using the automatic averaging and eye-tracking features of the proprietary device. Additionally to cSLO images, SD-OCT scans were used to prove the presence of RPD and the absence of other retinal pathologies in the study group, and, on the other hand, to prove the integrity of all retinal layers in the healthy control subjects (Fig. 1E). All scans were viewed and RPD were graded based upon the grading systems by Zweifel et al. and Querques et al.

Recently, a new approach to improve depth imaging by OCT, termed enhanced depth imaging (EDI) OCT, proved to be able to image the full thickness of the choroid reliably. Briefly, EDI-OCT uses the SD-OCT positioned closer to the eye than ordinary, such that a stable inverted image is produced. The net effect of this approach is that the sensitivity of the imaging in deeper layers of tissue is increased. According to recorded SD-OCT scans, EDI-OCT volume scans were obtained consisting of 61 scans centered on the fovea.

Automated computer software to calculate CT, choroidal vasculature thickness, or choriocapillaris thickness exists in prototype form only and has been tested exclusively in healthy subjects to date. However, they are not yet commercially available. As described previously by Tanabe et al., we constructed a CT map of the posterior pole that enabled us to determine the central CV and CT of the macula more reliably than by a single point-to-point measurement. Briefly, in each EDI-OCT scan, the cursor line marking the internal limiting membrane was moved manually to the outer border of the RPE. The cursor line originally marking the choroidal–scleral interface. Manual adjustment was performed in each of the 61 averaged EDI-OCT scans in study eyes and control eyes (Fig. 2A). Topographic choroidal maps were calculated automatically by analyzing all single scans with the Heidelberg Eye Explorer software. The
mean CT and CV values in different areas of the posterior pole were obtained from the choroidal maps using the previously described circular grid of the Early Treatment Diabetic Retinopathy Study (ETDRS). The ETDRS grid is an integrated feature of the Heidelberg Eye Explorer software and was centered on the fovea of the macular choroidal map (Fig. 2B). Comparisons between study eyes and control eyes were made for CV of the entire ETDRS grid area, CT, and CV of the sector of the ETDRS grid that was affected mostly by RPD, and CT and CV of the foveal sector of the ETDRS grid (Fig. 2C). As the sector area size of the ETDRS grid differs between inner and outer sectors, CV values of the sector that was affected mostly by RPD were normalized to the sector area.

**Reticular Pseudodrusen**

First and most frequently, RPD are noted between the superior part of the fovea and the superior temporal arcade. Funduscopically, they may appear slightly whiter or greyish compared to soft drusen. RPD may have a more punctate appearance closer to the fovea. Fundus autofluorescence (FAF) was performed to rule out any retinal alteration apart from RPD. Here, RPD can be detected as an area of decreased fluorescence surrounded by a faint halo of increased fluorescence adjacent to a network of reticular hypoautofluorescence.

To our knowledge, Arnold et al. first described the appearance of RPD in ICG as a pattern of hypofluorescent dots in the middle and late phase. In this study, identification of RPD depended on required characteristic features in cSLO FAF, IR, and SD-OCT imaging. For FAF imaging, RPD were defined as a regular network of uniform round or oval-shaped irregularities with a diameter ranging between 50 and 400 μm. Furthermore, lesions were characterized by a decreased FAF signal surrounded by mildly increased intensities. In IR imaging, RPD were identified as a pattern-like grouping of lesions varying in size with decreased reflectivity. For larger lesions, these images may be accompanied by a halo-like appearance exhibiting an increased IR signal in the center, surrounded by a decreased intensity. Querques et al. described this characteristic as a “target” aspect of RPD in IR similar to their appearance observed in FAF. In SD-OCT, RPD appear as hyperreflective material above the RPE in the subretinal space. RPD vary in shape and thickness, appearing as conical or flattened lesions.

**Statistical Methods**

The main outcome measures were presence and configuration of CWZ, topographic relation of CWZ to RPD area, CV of the complete ETDRS grid, CT and CV of the grid sector mostly
affected by RPD, and CT and CV of the foveal sector in both groups. Data were compiled and analyzed using Microsoft Excel (Microsoft, Inc., Redmond, WA). The Wilcoxon signed-rank test was used to compare CV and CT values of study eyes and control eyes. Statistical significance was set at $P < 0.05$.

**RESULTS**

Mean BCVA of the study eyes was 0.76 ± 0.18 (control group 0.87 ± 0.15). Mean refractive error was $-0.13 ± 1.10$ D for eyes affected with localized RPD and $+0.45 ± 0.95$ D for healthy control eyes. Incidence of systemic vascular diseases was 73.3% in the study group and 66.6% in the control group, which is not a significant difference ($P = 0.78$, adjusted G-test of independence). In the study group, 11 patients presented with exclusively RPD also in the fellow eye. Nine patients showed a CNV and 10 patients showed conventional soft drusen in the fellow eye. Age difference between both groups was not significant ($P = 0.41$, Student's $t$ test).

**Presence and Configuration of CWZ**

A CWZ was detected in 25 RPD (83.3%) and 12 control (40.0%) eyes. A stellate CWZ was seen in 10 eyes (40.0%) in the study group and 4 eyes in the control group (33.3%), in all of which the stellate pattern consisted of a vertical portion associated with multiple, smaller, triangular zones (Fig. 1A). A vertically oriented CWZ was observed in 11 eyes (44.0%) in the study group and 6 eyes in the control group (20%). An angle-shaped CWZ running through the optic nerve head and fovea was observed in 4 eyes (16.0%) in the study group and 2 eyes in the control group (16.6%). The relatively small numbers of each CWZ pattern did not allow us to make any correlation between the presence and localization of RPD, and particular patterns of CWZ. In total, CWZ could be identified in 25 subjects using vICG, and 10 of those CWZ could be identified using only vFA (Fig. 1C). Filling phases were comparable in both groups in vFA (study group mean 18.1 ± 3.5 seconds, control group 19.9 ± 2.9 seconds) and vICG (study group mean 18.7 ± 3.1 seconds, control group 20.4 ± 3.1 seconds). In all study eyes, RPD were identified clearly in late ICG and FA images as described above.

**Relation of CWZ to RPD Area**

In all study eyes, RPD were identified clearly in FAF and IR cSLO images as described above (Figs. 1B, 1D). Mean retinal area affected by RPD was 7.45 ± 2.25 mm$^2$. Interobserver comparison showed a mean difference of 0.25 mm$^2$. In 20 study eyes (66.6%), the ETDRS grid sector mostly affected by RPD was the outer superior sector. In 25 study eyes showing CWZ, localized RPD area was located fully or partly within the CWZ in 22 eyes (88.0%).

**CV and CT**

Localized RPD could be identified clearly in several scans as described above depending on the individual size of the area affected. In all included study eyes, RPD stages 1 and 2 were present, and at least one SD-OCT scan showed RPD breaking through the inner segment/outer segment boundary, corresponding to stage 3.29,30 Mean CV in the full ETDRS grid area was reduced significantly in the study group (4.49 ± 0.85 mm$^2$ in the control group, $P < 0.05$, Fig. 3A). Mean CV (Fig. 3B) and mean CT (Fig. 3D) in the ETDRS grid sector mostly affected by RPD were significantly diminished to 0.85 ± 0.38 mm$^3$/171 ± 61 μm, respectively, compared to 1.11 ± 0.38 mm$^3$/225 ± 54 μm in the corresponding ETDRS sector of the control eyes ($P < 0.05$). Mean foveal CV (Fig. 3C) and CT (Fig. 3E) values also were reduced significantly in the study group (0.14 ± 0.05 mm$^3$/174 ± 59 μm, respectively, versus 0.18 ± 0.05 mm$^3$/229 ± 58 μm, $P < 0.05$).

**DISCUSSION**

Choroidal circulation is the only source of blood and oxygen supply for the outer retina, and its proper functioning is crucial for maintaining the various functions of outer retinal cells. Obviously, any reduction in macular blood flow and changes in choroidal architecture would result in various pathophysiologic mechanisms. CWZ and choroidal volumes in patients with localized RPD have not been investigated before to our knowledge, and may give further insight in the pathogenesis of this AMD phenotype.

Comparable to the study of Giuffrè et al., who performed fluorescein angiography in 800 normal subjects and observed a well-outlined CWZ in 44.6% of all subjects,39 we found a CWZ in 40.0% of our healthy control group. In contrast, 85.3% of patients with localized RPD showed a well-demarcated CWZ in our study, which is comparable to reports on CWZ in patients with CNV due to AMD. For instance, Ross et al. found a correlation between the occurrence of CNV in AMD and the presence of CWZ.15 Of their patients presenting with exudative AMD 59% showed a CWZ, and in 91.7% of those patients the CNV extended directly out of the CWZ or its borders. The proximity of CNV to CWZ supports the hypothesis of ischemia having a key role in the development of CNV. Giovannini et al. studied choroidal filling patterns in 145 patients with or without CNV in AMD, and looked for a correlation between CWZ and CNV.14 They found a correspondence between CWZ and the site of CNV in 71% of cases. Mendrinos et al. recently evaluated the patterns of CWZ in exudative AMD using video-angiography and also described their relationship to CNV.28 They reported that CNV occurred within the CWZ in 44 of 50 (88%) patients. Stellate pattern of CWZ occurred most frequently, followed by the vertical and angled patterns.29 Comparing these data to ours, one then could postulate that the starting point of RPD development, similar to CNV development, is located in an area of poor blood supply, indicated by a CWZ.

Of all eyes with documented CWZ in the study by Giuffrè, the choroid lying nasally to the optic disc was involved in the area of CWZ in only 3% of the eyes.39 Interestingly, the area affected by localized RPD was never situated nasally of the optic disc in all our study eyes. Using merged three-field FAF images in over 400 eyes with RPD and GA due to AMD, Schmitz-Valckenberg et al. found that RPD were located most frequently superior to the fovea in the outer macula (98.0%), while RPD occurred least frequently nasally to the optic disc (51.0%).25 Our complementary data showed that RPD are located most frequently superior to the fovea not only in patients with late stage AMD, but also in patients showing exclusively localized RPD in a small retinal area. The question why this location is affected more frequently still is unclear. The fact that CT and CV values of the outer superior macular region are naturally higher than those of, for example, the outer inferior or outer nasal macular region might be a predisposing factor for RPD development.35 Curcio et al. hypothesized that preferential localization of RPD in the outer macula reflects a connection between RPD origin and rod physiology.4 Except for three patients, all study eyes showed localized RPD in the outer sectors of the ETDRS grid, which would support the theory that RPD originate rather from the rod than the cone metabolism.4
In patients with soft drusen maculopathy due to nonexudative AMD, Berenberg et al. could demonstrate that a larger extent of drusen significantly correlates with decreased foveolar choroidal blood flow, ergo, an inverse relationship between extent of drusen and choroidal blood flow. Studies by Grunwald et al. using Doppler flowmetry proved that decreased foveolar choroidal blood flow is associated with AMD and increased AMD severity. Moreover, it could be demonstrated that abnormal values of choroidal circulation predict the risk of progression of the disease, and that decreased choroidal circulatory parameters were detected before the formation of a CNV membrane. Xu et al. demonstrated an association between decreased choroidal circulation and a number of other risk factors for the development of AMD, for instance, RPE hyperpigmentation. Possibly, hypoxia modulates RPE metabolism and has a role in the formation of these RPE changes. The high incidence of CWZ in our study group and the close topographic relation to RPD areas suggested that choroidal blood flow also may be reduced in patients showing the RPD phenotype.

Association between AMD and systemic vascular diseases, such as systemic hypertension, have been investigated by many epidemiologic studies. Some of the studies could show an association, while others, such as the Beaver Dam Study, failed to establish an association. Metelitsina et al. reported that choroidal blood flow is approximately 16.7% lower in AMD patients with systemic hypertension than in AMD patients without it. Incidence of systemic vascular diseases was similar in both of our groups, which rules out a bias on choroidal measurements.

Histologic and OCT-based studies show strong evidence for choroidal thinning in advanced stages of AMD. However, evidence for choroidal thinning specifically attributable to early AMD has been inconsistent to date. With regards to previous reports, our data contributed to the insight that RPD are associated with choroidal thinning.

To our knowledge, Tanabe et al. were the first to report CV measurements by manually adjusting segmentation lines on EDI-OCT scans and calculating CT maps. Shin et al. recently reported a six radial scan protocol that generates a topographic map of CT and CV on a commercial SD-OCT device. The protocol requires only six radial B-scan segmentation line adjustments and takes 167 seconds to be performed, which reduces segmentation time significantly to a level that is acceptable for clinical use. For our scientific purposes in a limited number of patients, we aimed to avoid any interpolation processes and preferred to conduct the segmentation line adjustment manually in every scan.

Using the six radial scan protocol, Shin et al. reported a mean total CV of the entire ETDRS grid area of 7.72 ± 1.2 mm³ in a healthy population with the mean age of 46.2 years decreasing by 0.029 mm³ for each year of age, which is approximately in accordance with CV measurements of our control group and underlines the clearly reduced CV values of our study group. Using EDI-OCT, normal subfoveal CT was reported to be 287.6 ± 76 μm in patients with a mean age of 50.4 years. Given that subfoveal CT has been reported to decrease by 1.56 μm for each year, these values can be considered similar to those of our control group (229 ± 58 μm, mean age 75.4 ± 6.2 years) and additionally demonstrated a distinct decrease in subfoveal CT in our study group. Values of our control group also are in accordance with subfoveal CT measurements in normal subjects reported previously.

Querques et al. found that subfoveal CT as well as CT at different measurement points, except for the measurements at 5000 μm superior to the fovea, was reduced in eyes with only RPD, compared to eyes showing early AMD without RPD. They hypothesize, referring to the histopathologic report by Arnold et al., that RPD development and progression starts with a diffuse loss of small choroidal vessels and a diffuse choroidal thinning, and is followed by a fibrotic replacement.

**Figure 3.** (A-E) Box plots show CV and CT of study group (black) and control group (grey). Dots above box plot whiskers indicate outliers. Decreased CT and CV values in study group differed significantly from the values obtained in the control group in (A) through (E), as determined by the Wilcoxon signed-rank test. The length of the whiskers was defined by the data still in the 1.5x interquartile range, according to Tukey’s method. (A) CV of the entire Early ETDRS grid. (B) CV of the sector in the ETDRS grid that was affected mostly by reticular pseudodrusen compared to the corresponding sector in the control group. (C) CV of foveal sector in the ETDRS grid. (D) CT in the most RPD-affected ETDRS grid sector. (E) CT of foveal sector in the ETDRS grid.
and, thus, a slight thickening, particularly in the area of higher concentration of RPD. This theory is strengthened by our data as we confirmed a choroidal thinning at the very beginning of RPD development. Switzer et al. recently reported that patients with early AMD showing RPD as the predominant drusen type had a significantly thinner choroid, as compared to those early AMD patients without RPD. 7 In contrast to Querques et al. 10 and Switzer et al., 47 who performed CT measurements in RPD patients in a point-to-point manual caliper manner on a single line scan, which is limited to the subfovea or to a few points around the fovea, we used CT maps to generate more reliable values as well as volumetric data. Point-to-point measurements do not cover the entire macular area and can provide only limited information about changes in the entire choroid. Thus, CT and CV mapping are required for comprehensive assessment of the choroid. Unlike Querques et al. 10 and Switzer et al., 47 who compared their measurements to other early AMD patients, we compared our data to healthy age-matched test persons. Furthermore, one crucial characteristic of our study group was that the RPD phenotype was restricted to a small retinal area to capture the early phase of RPD evolution. Patients suffering from AMD often show RPD distributed across a large part of the posterior pole. In contrast with previous studies, we investigated only patients who showed distinct RPD in a relatively small area of the macula as we wanted to ensure to include only patients showing the "startpoints" of the RPD phenotype. The topographic relationship between the site of RPD areas and CWZ suggested that choroidal ischemia may be a predisposing factor for the development of RPD. Although AMD is not an acute ischemic condition, it might involve a chronic vascular insufficiency.

Our study showed that the presence and localization of CWZ, as well as reduced choroidal volumes and thicknesses, are associated with localized RPD. Our study has several limitations. The series presented comprised a relatively small number of patients. However, one should consider the strict inclusion criteria for the RPD and age-matched control groups. vICG is known to visualize choroidal circulation clearly and allows for readily identifying CWZ; however, this technique does not offer quantitative measurements of choroidal blood flow. Furthermore, a weakness of this technique is that it requires subjective interpretation of the angiogram to determine whether a CWZ exists and where its boundaries are localized. Our data could be compared to other techniques, like Doppler flowmetry, which measures a relative blood flow rate, or Doppler OCT, which is capable of providing an absolute blood flow rate. 17,18,20–22,26,51 A general limitation in drawing conclusions from CT and CV measurements is the variation of the choroid being a complex system influenced by intraocular and perfusion pressures as well as regulatory mechanisms of various vasoactive agents. We did not perform a detailed segmentation of each choroidal layer including choriocapillaris and choroidal vasculature as reported recently in a small, healthy population. 32 Nevertheless, a more precise delineation of the different layers of the choroid may reveal further insights in RPD pathology that are difficult to observe when the entire choroid is summed. The software displays cSLO images alongside the OCT scans, which unmasks the readers when manually adjusting cursor lines in OCT scans regarding the presence of RPD and represents a further limitation. With no automated computer software commercially available to calculate choroidal thickness maps, measurements had to be performed manually. Our study cannot answer the question of cause-effect relationship. Based on our study, we cannot conclude that CWZ or reduced CV causes the development of RPD, or that the presence of RPD may promote the development of CWZ and reduces CV. Further studies are needed to prove a causative relationship.

To our knowledge, this is the first study to identify CWZ and to assess CV maps in patients with localized RPD. Longitudinal studies will show if the progression of RPD stages, growth of RPD-affected retinal areas, and direction of RPD spread depend on the presence and localization of CWZ.

Acknowledgments

Disclosure: F. Alten, Heidelberg Engineering (C), Novartis (C); C.R. Clemens, Heidelberg Engineering (C), Novartis (C), Bayer (C); P. Heiduschka, Novartis (C); N. Eter, Heidelberg Engineering (C), Novartis (C), Bayer (C), Sanofi Aventis (C), Allergan (C), Bausch and Lomb (C)

References

15. Ross RD, Barofsky JM, Cohen G, Baber WB, Palao SW, Gitter KA. Presumed macular choroidal watershed vascular filling, choroidal neovascularization and systemic vascular disease in