Image Registration and Multimodal Imaging of Reticular Pseudodrusen

Mahsa A. Sohrab,1,2 R. Theodore Smith,2 Hani Salebi-Had,1 SriniVas R. Sadda,1 and Amani A. Fawzi1

PURPOSE. To characterize reticular pseudodrusen (RPD) by using a point-to-point comparison of the reticular pattern on infrared reflectance (IR), autofluorescence (AF), and red-free (RF) images registered with en face sections of the choroid from spectral domain optical coherence tomography (SD-OCT) scans.

METHODS. A cross-sectional, retrospective study of all patients with the diagnosis of AMD who presented to the Doheny Retina Institute between December 2007 and November 2009 was conducted to identify patients with RPD. IR, AF, and RF images were obtained using confocal scanning laser ophthalmoscopy and were manually registered to OCT choroidal sections to study the location of RPD. The main outcome measured was point-to-point localization of RPD across multiple imaging modalities.

RESULTS. Of the 153 patients with AMD, 51 had RPD. In all 51 patients (97 eyes), RPD appeared as areas of hypoeufluorescence and hyporeflectance on AF and IR imaging, respectively, and as hyporeflective interfacing networks on RF. Reticular lesions on AF, IR, and RF images consistently localized with stromal regions between large choroidal vessels on registered en face choroidal sections. In contrast, outer retinal changes and subretinal deposits tended to localize immediately adjacent to the RPD.

CONCLUSIONS. Point-to-point correlation of registered IR, AF, and RF images consistently localized the reticular pattern to the intervascular choroidal stroma on en face OCT sections. In contrast, subretinal deposits and disturbances of the inner outer segment on OCT did not colocalize with the RPD, and may represent secondary mechanical or biologic disturbances in the overlying RPE and outer retina. (Invest Ophthalmol Vis Sci. 2011;52:5743–5748) DOI:10.1167/iovs.10-6942

Since the initial description of reticular pseudodrusen (RPD) on blue light photography,1 numerous researchers have attempted to identify the location of these abnormalities and gain insight into their pathogenesis using advanced retinal imaging technologies.7–9 Histopathologic correlation from the initial report revealed a significant loss of the small vessels of the middle choroidal layer and increased spacing between the large choroidal veins, leading Arnold et al.2 to postulate that fibrotic replacement of the choroidal stroma and loss of vascularity was responsible for development of RPD.

Smith et al.6 used automated image registration to study the appearance of RPD in various imaging modalities, and showed lesion-to-lesion correspondence between autofluorescence (AF), infrared reflectance (IR), indocyanine green angiography (ICG), red-free (RF), and color fundus photographs. Based on their findings, they proposed that RPD are related to alterations in the RPE and the inner choroid.10

Studies using Heidelberg Spectralis spectral-domain optical coherence tomography (SD-OCT) have suggested that RPD may correlate with granular hyper-reflective deposits in the subretinal space.7,8 These OCT findings were correlated to previously published histopathologic findings in three unrelated eyes showing subretinal deposits, of whom none had a clinical diagnosis of RPD.7,9

Advances in optical imaging technologies have allowed improvements in visualization of the choroid. Margolis and Spaine,10 Fujiwara et al.,11 Spaide,12 Imamura et al.,13 and Ikuno et al.14 advocate a technique for improving sensitivity on the choroidal side of the Heidelberg Spectralis SD-OCT B-scan by bringing the inverted conjugate image into view, an approach called enhanced depth imaging. Some SD-OCT devices, however, have a less steep drop in sensitivity from the vitreous to the choroidal side, and averaging of multiple B-scans alone appears sufficient to provide excellent visualization of the choroid. In a recent study, noninverted high definition (HD) single raster line images (an average of 20 B-scans) on the Cirrus HD-OCT device (Carl Zeiss Meditec Inc., Dublin, CA) were found to be sufficient to measure choroidal thickness in normal eyes.15 While many studies have focused on measuring choroidal thickness and have showed age-related choroidal thinning using these technologies,10–15 to our knowledge, none have used en face cross-sections of the choroid (C-scans) for detailed evaluation of choroidal pathologic changes.

The goal of the present study was to evaluate choroidal changes in eyes with RPD using SD-OCT imaging. In addition, to address the apparent controversy related to the exact location of RPD lesions arising from previous conflicting reports,7,9,16 we performed point-to-point correlations between RPD lesions on IR reflectance and registered SD-OCT B-scans, as well as SD-OCT en face choroidal and retinal sections.

METHODS

Study Population

The study was approved by the Institutional Review Board at the Doheny Eye Institute and adhered to the tenets set forth by the Declaration of Helsinki. A retrospective review of all patients diagnosed with AMD at the Doheny Eye Institute between December 2007 and November 2009 identified 153 patients who had undergone IR, AF,
and SD-OCT imaging with the Spectralis HRA + OCT (Heidelberg Engineering Inc., Heidelberg, Germany). Some patients had RF images taken with the Spectralis HRA, although the majority had RF images obtained as part of the standard fluorescein angiography (FA) protocol, which used the Topcon TRC-50IX (Topcon Medical Systems Inc., Paramus, NJ). Considering the entire portfolio of imaging studies for each patient, and applying the strict criteria for the presence of RPD described in the Definitions section below, we identified 51 patients (97 eyes) with RPD. Twenty-nine (55 eyes) of the 51 patients also had volume SD-OCT macular cube scans obtained with the Cirrus high-definition SD-OCT device (HD-OCT; Carl Zeiss Meditec Inc., Dublin, CA). We included both eyes for each patient in this study, as long as they had available and good quality images. Eyes were excluded if they had undergone retinal surgery, or if images were not obtained because of extensive media opacities.

**Image Acquisition**

Twenty-nine patients underwent scanning with the Cirrus HD-OCT Model 4000 device, using superluminescent diode (SLD) at 840 nm, which achieves 5 μm of axial and 15 μm of transverse tissue resolution. The device captures 27,000 A-scans per second at 2 mm of depth, and the images were viewed with the latest Cirrus HD-OCT software (v 5.0; Carl Zeiss Meditec Inc.). As part of the standard Cirrus imaging protocol at the Doheny Eye Institute Ophthalmic Imaging Unit, all eyes undergo two scanning protocols, a five-line raster consisting of 4096 A-scans for each of the five B-scans, and a 512 × 128 macular cube volume scan consisting of 128 equally spaced horizontal B-scans (each composed of 512 A-scans) over a 6-mm square grid. The line scanning laser ophthalmoscope (LSLO) feature also obtained a registered OCT fundus image for each data cube. The Cirrus OCT imaging protocol also requires photographers to repeat OCT volume scans if the summed OCT projection image suggests that significant motion artifact is present. Cirrus OCT scans that were free of motion artifact were selected for this study.

IR (790 nm), RF (488 nm), and AF images were obtained using the Heidelberg Spectralis HRA + OCT confocal scanning laser ophthalmoscope and SD-OCT device (Heidelberg Engineering Inc.). For AF images, blue laser light at 488 nm was used for illumination and a barrier filter at 500 nm was used to limit captured light to autofluorescent structures. Spectralis SD-OCT images were obtained by using a volume cube 512 × 37 × 37 (over a 6-mm square grid) averaging nine scans for each B-scan. For a subset of patients, RF images were obtained using the Topcon TRC-50IX (Topcon Medical Systems Inc.).

**Definitions**

The identification of patients with evidence of RPD was based on the recognition of characteristic features on various imaging modalities as defined in previous reports.6 Evidence of RPD on RF was defined by the presence of light, interlacing networks ranging from 125 to 250 μm in width. RPD on AF was defined by the presence of clusters of ill defined, hypoautofluorescent lesions interspersed against a background of mildly increased AF occurring in regular and well defined array. RPD on IR was defined as groups of hyporeflective lesions interspersed against a background of mild hyper-reflectance.

**Analysis Protocol**

Point-to-Point Registered OCT B-Scan Comparisons. Spectralis OCT scans were reviewed for all 51 patients to take advantage of the retinal tracking and precise Tru-Tracking registration capability, which allows point-to-point correspondence between the AF, IR, and RF images and the OCT B-scans.

En Face OCT Choroidal and Outer Retinal Sections. The following analysis was performed on each of the 55 eyes of 29 patients who also underwent Cirrus OCT imaging. OCT volume scans (512 × 128 macular cubes) obtained on Cirrus HD-OCT were reviewed on the Cirrus version 5.0 software using the advanced visualization feature. As previously described,16,17 the RPE feature was used to obtain en face slices, or C-scans, which were contoured based on each patient’s RPE curvature. The horizontal section (slab thickness) was adjusted to ensure that the RPE band or sclera were not included in any of the C-scan slabs. The inner aspect of the slab feature was placed at a fixed distance below the RPE band, and then en face choroidal slabs of variable thickness (ranging from 20 to 60 μm) were generated and reviewed for every patient. By using the RPE slice overlay feature, these slabs were reviewed as registered to the Cirrus IR fundus image. Additional en face sections including only the inner segment/outer segment (IS/OS) junction and outer nuclear layer were also obtained and overlaid onto the Cirrus IR image for review.

**Manual Registration of En Face OCT Sections to Fundus Images.** Retinal vessel crossing points were used as invariant landmarks to allow manual registration of the OCT fundus (projection) image with the cSLO IR and AF and RF images (obtained with either the Spectralis cSLO or Topcon camera). GIMP, a freely available GNU Image Manipulation Program (v 2.6; available from http://www.gimp.org) was used to align and register the images.

New drawing layers were then created and the paint brush tool was used to manually segment individual RPD lesions on the IR images, which were then superimposed to the Cirrus OCT C-scan slabs to look for correspondence between the RPD lesions on IR images and structures on the OCT. C-scans from the 40- to 60-μm depth slabs were used for all patients except those with severe choroidal atrophy (choroidal thickness <70 μm), in which case the 20- to 30-μm depth slabs were used. Assessments were made by one grader (MAS) and then reconfirmed by a second independent senior grader (AAF).

**Transitional Overlays of Registered En Face OCT Sections and Fundus Imaging.** OCT sections of the choroid reconstructed, as previously described, were registered to IR, AF, and CF images (for cases in which patients had prominently visible choroidal vessels on CF). Transitional overlays were created in GIMP using increasing levels of opacity of the overlying image.

**RESULTS**

**RPD Patient Characteristics**

Of the 51 patients included in the study, 80% (41/51) were female, with average and median ages of 83 years (range, 66–102). A total of 97 eyes were analyzed, all of which showed evidence of RPD based on the criteria outlined in the Methods section. The Cirrus HD-OCT subset (29 patients/55 eyes) was similar to the total study cohort, with an 83% (24/29) male ratio and an average and median age of 83 years (range, 55–95). Of the total eyes reviewed, 44% (43/97) had late AMD (neovascular or atrophic) and 56% (54/97) had early AMD changes including soft and hard drusen with or without pigmentedary changes. Of the eyes with late AMD, 84% (36/43) exhibited the neovascular form of AMD, which was bilateral in 44% of these eyes. Atrophic late-stage AMD was found in 16% of eyes (7/45) of late-stage patients, with 40% bitalerality.

**Reflectance and Autofluorescence Imaging**

Reticular patterns appeared as areas of hypoautofluorescence and hyporeflectance on AF and IR, respectively, and as light interlacing networks on RF. Registered AF, IR, and RF images displayed overlapping areas of reticular involvement, with the reticular lesions occupying the largest footprint area on IR imaging. IR imaging also revealed reticular patterns in the central macular area, which were not visible on AF and RF images (Fig. 1).

**OCT Imaging**

Point-to-Point Comparisons of Registered OCT B-Scans and Fundus Imaging. Comparisons were made between OCT B-scans derived from the Spectralis and Cirrus and respective AF, IR, and RF images from each patient. On Spectralis HRA + OCT, 90% (46/51) of patients had patchy hyper-reflective alterations at

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the inner aspect of the RPE layer, consistent with subretinal deposits. These hyper-reflective abnormalities extended from the photoreceptor–RPE junction to the photoreceptor IS/OS junction. We found that RPD lesions as identified on IR often corresponded to areas with no apparent disruption or disturbance to the retina or RPE on OCT B-scans (Figs. 1, 2).

Similarly, the subretinal lesions as identified on OCT did not consistently show a one-to-one correlation with RPD lesions on AF, IR, and RF images (Fig. 2). Rather, these subretinal lesions consistently corresponded with areas immediately adjacent to the RPD lesions themselves on IR (Fig. 2).

**Point-to-Point Comparisons of Registered En Face OCT Sections and Fundus Imaging.** Sections of choroid of varying thickness evaluated en face (OCT cross-sections) were viewed as OCT slabs registered to IR images on Cirrus HD-OCT and were manually registered to fundus imaging. RPD lesions marked on registered IR, AF, and CF images followed the pattern of the underlying choroidal stroma on en face OCT sections (Figs. 3, 4). Individual reticular lesions were found to overly choroidal stroma, closely abutting (but definitely not overlying) larger choroidal vessels (Figs. 3, 4). Areas of hyper-reflective subretinal deposits and associated IS/OS disruption frequently occurred directly ad-jacent to RPD lesions (Fig. 3). En face OCT sections through the IS/OS junction and subretinal space yielded a map of the distribution of these small hyper-reflective deposits. By comparing this map to the IR image, it became clear that the subretinal lesions do not correspond to the entire RPD pattern (not shown).

**Comparisons of En Face OCT Sections and Fundus Imaging through Transitional Overlays.** When reviewing transitional overlays of varying opacities of choroidal en face images obtained on OCT and manually registered IR, AF, and CF images, similar associations of reticular patterns with choroidal vessels were noted. These images revealed that groups of reticular lesions closely followed the outline of the large choroidal vessels (Figs. 4, 5). Individual RPD lesions abutted and appeared to line up along the edges of the choroidal vessels.

**DISCUSSION**

Using en face OCT sections of the posterior pole, we were able to map RPD lesions across imaging modalities and illustrate their alignment with the intervascular choroidal stroma, and not with subretinal deposits or areas of IS/OS disruption. To our knowl-

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**FIGURE 1.** Left eye of a patient with RPD imaged on a Heidelberg Spectralis OCT device. Autofluorescence (AF, left), infrared (IR, center), and horizontal OCT B-scans (right) are shown. Dashed horizontal lines through areas with RPD (IR) are registered to the horizontal OCT B-Scans on the right, labeled (A) and (B), respectively. The registered vertical marker within each box shows that individual RPD lesions do not correspond to RPE or inner segment/outer segment changes on OCT, and instead lie adjacent to them. This is highlighted by the magnified insets. Comparing the AF and IR panels highlights how RPD lesions are better seen on the IR, especially centrally and temporally.

**FIGURE 2.** Left eye of a patient with RPD imaged on a Heidelberg Spectralis OCT device revealing inner segment/outer segment (IS/OS) changes in areas between RPD lesions. Infrared (IR, A) and registered horizontal OCT B-scans (B) corresponding to the horizontal dashed line on IR are shown. The light vertical markers within each box on the IR are registered to individual RPD lesions and do not reveal IS/OS changes on OCT B-scans (light vertical lines on OCT). The dark vertical markers within each box on the IR fall between individual RPD lesions, which in turn correspond to areas of IS/OS changes on OCT scans (dark dashed lines on OCT). Magnified insets highlight the areas of interest.
edge, there have been no previous reports using en face OCT sections of the choroid to facilitate these correlations.

Given the controversies surrounding the location of RPD lesions, and the lack of consensus, we used en face sections (slabs) on Cirrus HD-OCT, overlaid on Cirrus IR images, to determine whether the reticular patterns could be explained by choroidal alterations as originally postulated.\(^2\) Hyporeflective (IR) and hypoautofluorescent (AF) RPD lesions consistently colocalized to stromal regions, whereas large choroidal vessels on OCT sections consistently colocalized with hyper-reflective (RF) and hyperautofluorescent (AF) areas between individual reticular lesions, and with occasional subretinal deposits (Fig. 3).

Subretinal hyper-reflective deposits on OCT were recently described in RPD, and were theorized to be an essential component of this disease.\(^7,8,18,19\) However, our review of IR images and registered OCT B-scans on Spectralis through point-to-point localization of individual RPD lesions—a technique not described in previous studies\(^7,8\)—did not show consistent association of RPD lesions with any IS/OS, subretinal, or RPE alterations (Figs. 1, 2, and 3). The True-Tracking registration feature of Spectralis HRA + OCT offers the distinct advantage of accurate point-to-point correlations between IR, AF, and RF imaging and OCT. The requirement to average multiple scans to achieve optimal image quality, however, limits the scanning density when obtaining volume Spectralis HRA + OCT cube scans, which may limit the ability to map small lesions when performing en face (C-scan) reconstructions.

To gain a better understanding of the overall mapping of these outer retinal lesions throughout the posterior pole in RPD, and to confirm the findings noted on Spectralis HRA + OCT, we studied volume Cirrus OCT scans (6- x 6-mm cubes) on a subset of patients. En face sections through the outer retina and subretinal space generated maps of these subretinal hyper-reflective deposits, which, overlaid onto the IR fundus image, confirmed that the

![Figure 3](image_url)

**Figure 3.** Individual RPD in the right eye of a patient correlates to the choroidal stroma on OCT en face imaging. Contrast-enhanced infrared (IR) image from the right eye of a patient with RPD (A), horizontal OCT B-scan (B), and en face pseudocolorized choroidal section (C) are displayed. Dashed green horizontal line in (A) and (C) is registered to the horizontal OCT B-scan. The registered green vertical marker on IR overlies an individual RPD lesion, which in turn corresponds to an area adjacent to inner segment/outer segment (IS/OS) change on OCT (green vertical line, B) and to choroidal stroma on en face imaging (green vertical marker, C). The registered red vertical marker on IR lies adjacent to the selected RPD lesion, which in turn corresponds to IS/OS change on the horizontal OCT B-scan (dashed red line, B) and to a large choroidal vessel on en face imaging (red marker, C). The areas of interest on the IR and OCT B-scan are highlighted as magnified insets.

![Figure 4](image_url)

**Figure 4.** Transitional overlay of choroidal en face OCT scan onto the infrared image of the right eye of a patient with RPD shows the alignment of RPD lesions along choroidal vessels. Infrared (IR) image of a patient with RPD obtained on the Heidelberg Spectralis device (A) was manually registered to the en face choroidal OCT section reconstructed on the Cirrus device (D). The en face choroidal scan was pseudocolored on GIMP. Transitional overlay of the en face OCT section onto the IR at increasing opacities (B and C, respectively) reveals alignment of RPD lesions with large choroidal vessels, as seen in the magnified inset from (C).
extent of these outer retinal lesions does not explain the entire RPD pattern in the posterior pole. This is also consistent with the finding that subretinal deposits cannot account for the impaired filling on FA and ICG seen in RPD patients and the interconnected, lacy appearance of well defined reticular patterns on blue light and RF imaging. Until recently, the only histopathologic report of RPD implicated the choroid but did not include sections of the neurosensory retina.2 Since the initial report, there have been reports of histopathologic findings from three patients with subretinal drusenoid deposits, two of whom had a documented history of AMD but no history of RPD.7 Notably, these were not the same patients in whom the OCT imaging was performed,7,9 and therefore the clinicopathologic correlation of RPD was not possible.

One report on eyebank eyes that included eyes with RPD noted improved visualization of the reticular pattern with removal of the neurosensory retina.20 Most recently, however, Sarks et al.21 noted the presence of subretinal deposits on histopathology from the eye of a patient with RPD, cautioning that these deposits have also been found in patients with AMD but without the presence of RPD on fundus imaging. Correlation of histopathology from a patient with confirmed RPD to OCT imaging also noted an association of a subtype of RPD with subretinal deposits, but it was noted that derangement of the RPE because of underlying fibrosis of the choroids could lead to the accumulation of photoreceptor outer segments above the RPE.22

To further clarify the location and significance of the outer retinal changes in RPD, we examined the correlation of these lesions with cross-sections of the choroid. We found that subretinal hyper-reflective deposits overly large choroidal vessels and do not correspond with areas of RPD on IR, but instead lie immediately adjacent to the RPD lesions on IR (Fig. 3). This confirms the findings seen on Spectralis HRA + OCT (Figs. 1, 2). We believe that the apparent contradiction of these findings with previous reports of OCT in eyes with RPD7,8 is related to the density of the OCT scans used in those studies (seven line scans through the macular area, each of which comprises up to 100 averaged scans, 30° transverse B-scan with a total of 768 A-scans). Low density scans, as obtained in those studies, would not allow for detailed point-to-point registration of an entire zone of RPD. In contrast, our approach using a high-density map (Cirrus macular cube; 6 × 6 mm), allowed us to create an en face map (slab) of the outer retina and the choroidal vasculature in the entire macula. We feel our approach provided a more consistent and precise method for intermodality correlations, and therefore revealed findings that were not previously noted.

The demographics of our RPD patient population was consistent with previous published studies and confirms an older population (average age, 83 years) with a strong female preponderance (80%).5,23 Interestingly, while the results of a comprehensive epidemiologic review of RPD did not find an increased incidence of neovascular as opposed to atrophic late-stage AMD in their population,23 our RPD study population had a significant preponderance of patients with neovascular AMD (83% neovascular vs. 17% atrophic), similar to the several other published reports.2,4,6

The appearance of RPD as hyporeflective, interfacing networks on RF and as areas of hyporeflectance and hypofluorescence on IR and AF, respectively, was consistent with the findings and definitions of Smith et al.6 Their study found that IR was most sensitive for the detection of RPD, and used IR to show the presence of RPD crossing the central macula.6 We noted a similar sensitivity of IR for RPD, and found that IR images revealed more extensive RPD involvement than AF and RF images (Fig. 1).

It is intriguing to speculate on the pathologic correlates of RPD as it relates to the choroidal stroma. Gass et al.24 reported a reticular pattern of fundus appearance in two patients with systemic non-Hodgkin lymphoma that presented a "reticular pattern of yellow-orange flecks" that, similar to RPD, did not show staining in early- or late-phase fluorescein angiography. Gass et al.24 postulated that the fundus appearance, which was a precursor for systemic disease in these patients, represented transient infiltration of the choroidal stroma or sub-RPE space by lymphoma cells, potentially associated with an inflammatory response and subsequently replaced by areas of fibrous metaplasia. The similarity of
the imaging findings in these cases to RPD suggest that RPD might be associated with inflammatory or atrophic vascular changes of the choroidal stroma.

The pathogenesis of RPD remains unknown. A recent epidemiologic study revealed that, unlike drusen and pigmented changes found in AMD, RPD lesions are associated with increased mortality independent of comorbidities, such as hypertension, diabetes, and cancer, which is suggestive of an unidentified systemic process.

The choroidal location of the pathology is supported by the fact that the highest concentration of components of the alternate and classical complement pathways, including C5b-9, are in the choroid, with significantly lower levels identified in the RPE and neural retina. Therefore, assembly of the membrane attack complex depends on the choroid, suggesting that local complement activation at the level of choroid is associated with AMD pathogenesis. These researchers have shown that the choroid is capable of elaborating all components of the complement pathway, a characteristic not shared by the RPE or the neural retina. An inflammatory process with complement activation within the choroid, followed by possible atrophic vascular compromise and fibrous replacement can yield the characteristic appearance of RPD on RF, IR, and AF imaging. In addition, this fibrous replacement would explain the impaired filling reported in patients with RPD on FA and ICG imaging, likely secondary to blocked fluorescence. This would also explain our OCT findings, where choroidal vessels have been replaced with “choroidal stroma” on choroidal OCT C-sections, while secondary (ischemic, metabolic, or mechanical) disruption of overlying or adjacent RPE could account for the reported subretinal deposits on OCT.

Our study was limited by several factors. First, only a subset (29) of the 51 patients had Cirrus OCT scanning, and therefore choroidal slab analysis was not possible for all subjects. Second, we had no examples of histopathology to correlate to the morphologic changes noted on en face choroidal sections in our population. Third, this was a retrospective analysis and is subject to ascertainment bias and other potential unknown confounders. However, the reproducibility and consistency of our findings among our patients, and the similarity of our findings to those previously reported on histopathology from a patient with known RPD would appear to support our conclusions. The strengths of our study include the use of en face examinations of the choroid, in combination with high density scans, and cross validation of our findings through point-to-point localization across various imaging modalities, combined provided a greater resolution and mapping of structural changes in the retina, RPE, and choroid compared to previous reports.

In conclusion, using en face OCT imaging and precise image registration, we provide evidence suggesting that the arrangement and pattern of RPD is most closely related to the choroidal stroma and the choroidal vasculature. In addition, our findings suggest that the previously reported RPE derangements and subretinal deposits may be secondary pathologic changes that do not consistently correlate with the RPD pattern, and only occasionally occur in these choroidal lesions. Our results yield a better understanding of the reticular pattern and provide a comprehensive explanation for the variety of imaging findings in this condition. Additional histopathologic studies are needed to elucidate the pathology of these choroidal stromal changes and the nature of the RPE and photoreceptor derangements.

References