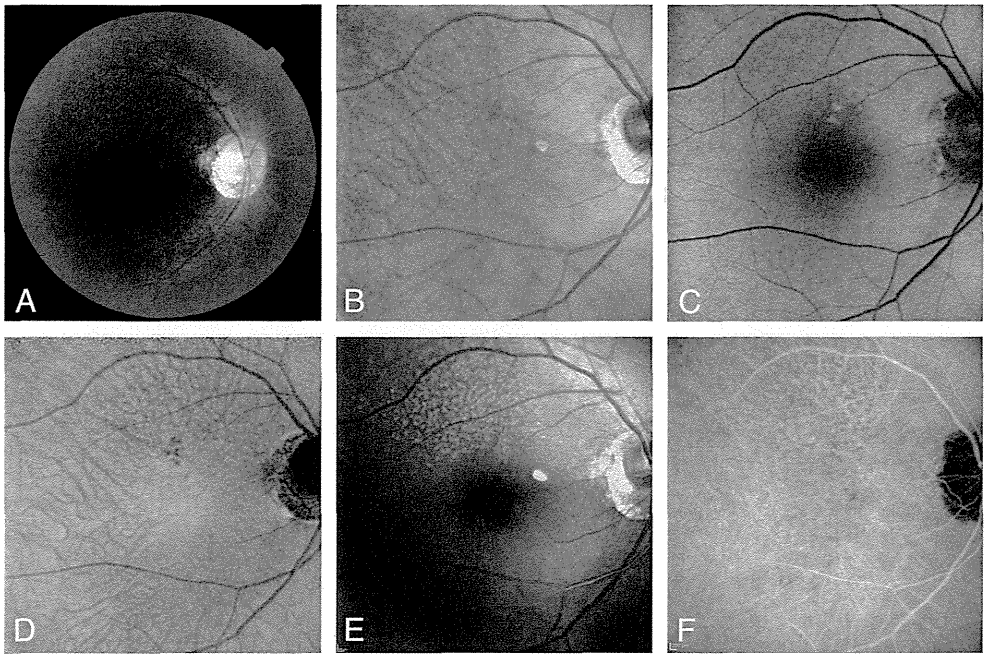

Fig. 3. Distributions of reticular lesions in different imaging modalities in an eye with RPD. **A**. Blue channel of color fundus photography. IR (**B**), FAF (**C**), NIR-FAF (**D**), CBR (**E**), and IA (**F**) images.

of ill-defined lesions with decreased reflectance, but soft and hard drusen occasionally have a relatively similar appearance in IR images, with decreased brightness compared with the background; this may lead to decreased specificity of IR for RPD detection.

The blue channel of color fundus photography and CBR had specificities of 100%. At short wavelengths, blue light is preferentially absorbed by melanin, and therefore, the RPE offers a darker background.[14] Typical soft and hard drusen appear dim in the blue channel because blue light is absorbed by the overlying RPE. In contrast, Zweifel et al[7] demonstrated that the location of RPD corresponds to abnormal material lying above the RPE. Thus, RPD are selectively visible in the blue channel of color fundus photography or CBR, leading to better specificity for RPD detection. The subretinal location also may block reflectivity from the RPE and account for its characteristic dark reticular appearance

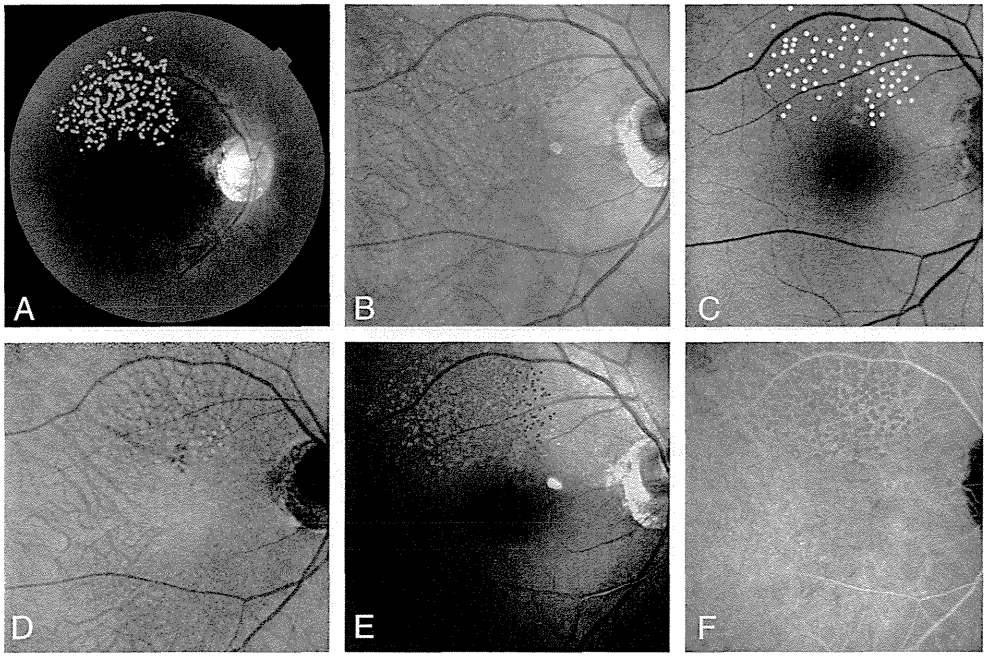

Fig. 4. Areas of reticular lesions in different modalities of the same eye in Figure 3. Reticular lesions are manually marked as dots. On IR imaging, reticular lesions are seen in larger areas, compared with other modalities.

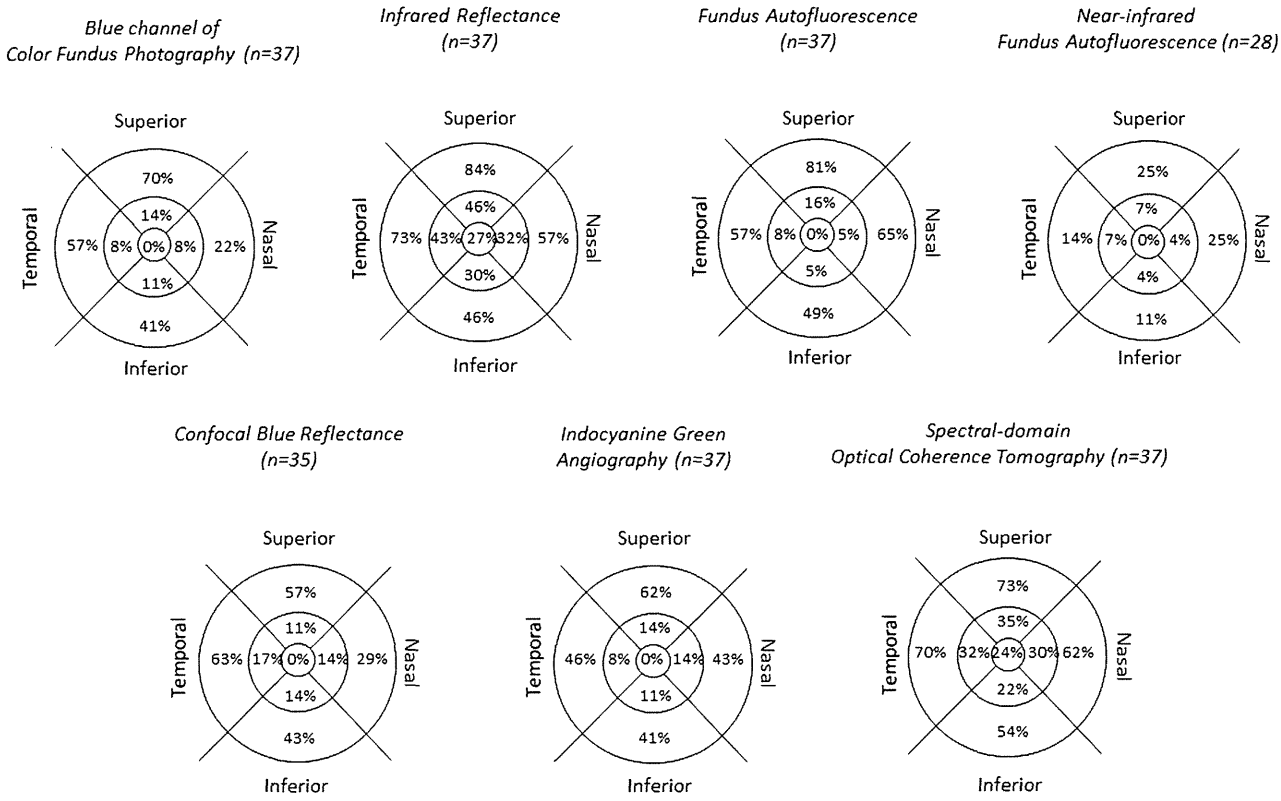

Fig. 5. Distributions of RPD in different imaging modalities. The diameter of the inner, middle, and outer circle is 1 mm, 3 mm, and 6 mm, respectively. Reticular pseudodrusen are most frequently seen in outer superior subfield of macula in all modalities. Of the seven modalities, only IR and SD-OCT can detect RPD within the center circle area.

on FAF.[9] However, compared with IR, color fundus photography or CBR imaging may be hampered to a greater extent by media opacity; therefore, these imaging methods had lower sensitivity than IR.

Recently, it was shown that RPD could be clearly differentiated from typical soft drusen using SD-OCT. Zweifel et al[8] reported that in eyes with late AMD, RPD were found much more frequently using SD-OCT than by examining the blue channel of color fundus photography. The present study showed that SD-OCT had the highest sensitivity and high specificity for RPD detection, suggesting that SD-OCT is the most useful method for diagnosing RPD among the seven modalities. However, it has been reported that RPD fades in the vicinity of CNV but often remains identifiable outside the macula.[4] Thus, especially in eyes with CNV, SD-OCT scans should be obtained over a wide area that includes the outside of the macula to accurately detect RPD. In addition, several studies have revealed that RPD lesions are seen most frequently in and near the superior arcades (Figure 2)[6,12]; thus, the imaging protocol should include this area for RPD detection. In a study by Zweifel et al,[8] 31 B-scans were obtained within a 20° × 25° rectangle. In our study, horizontal and vertical line scans through the fovea center were obtained at a 30° angle, followed by serial horizontal scans with an examination field size from 30° × 10° to 30° × 25°.

Middle- to late-phase IA hypofluorescence has been observed in RPD,[13] and Smith et al[11] verified that IA lesions were precisely collocated with RPD and lesions in reticular FAF. They also reported that all eyes with RPD also had IA lesions, although the sample size was small (n = 9).[12] In the current study, we examined a larger number of eyes using IA and found that IA had a specificity of 100% and relatively high sensitivity for detecting RPD. Thus, IA also may be a useful method for RPD detection.

Recently, NIR-FAF results have been reported for various macular diseases.[15,16] Near-infrared FAF is believed to originate from melanin and/or compounds closely related to melanin,[15] and NIR-FAF images show an area of high autofluorescence around the fovea in a normal retina, corresponding to the higher distribution of melanin in the foveal RPE. However, the produced signal is 60 to 100 times less intense than that obtained with FAF imaging,[15] leading to a relatively dark background. On NIR-FAF images, RPD appears hypofluorescent with low contrast with the dark background, and the current study found that NIR-FAF had the lowest sensitivity for detecting

RPD. In addition, this modality also had the lowest intergrader agreement, indicating there is high subjectivity in diagnosing RPD. Thus, NIR-FAF may be less useful for diagnosing RPD.

Reticular pseudodrusen were most frequently detected in the superior outer macula in all modalities, consistent with Smith et al.[12] They reported that the region involved by the IR lesions in 52% eyes covered the central 1-mm-diameter zone, although the FAF lesions or color fundus images never involved the central zone,[12] similar to the results of the current study. In addition, our study showed that SD-OCT also could detect RPD within the central zone. These results suggest that IR and SD-OCT imaging may be necessary for the visualization of RPD in the central macula.

It may be challenging to diagnose RPD using a single imaging method because the characteristic changes associated with RPD are often subtle, and other alterations may result in overlooking or misidentifying RPD. In the current study, we showed that the percentage of eyes with reticular pattern differed among the seven imaging modalities. For detecting RPD, SD-OCT and IR showed the highest sensitivity (>90%), and blue channel of color fundus photography, CBR, IA, and SD-OCT showed the highest specificity (>98%). Thus, SD-OCT is the most sensitive and specific for detecting RPD. In addition, IR and SD-OCT could detect RPD in more subfields compared with other modalities. However, several false-positives and false-negatives were also seen even using SD-OCT. Thus, RPD detection should ideally be performed using more than one imaging modalities. The findings of the current study suggest that IR and SD-OCT, which showed high sensitivity, may be useful for screening RPD and that the blue channel of color fundus photography, CBR, and IA, with specificities of 100%, may be useful for confirming RPD diagnosis.

Our study had several limitations. In addition to its retrospective nature and relatively small sample size, the scanning range of SD-OCT did not necessarily cover the entire area of other SLO imaging techniques; this may have resulted in underestimating the sensitivity of SD-OCT. However, we found similar results by reviewing SLO images only corresponding to the area imaged by SD-OCT. Furthermore, RPD is occasionally present only on the nasal side of the optic disk, and our study may have missed such eyes. Despite these limitations, RPD was found in approximately 20% of the patients with newly diagnosed AMD using multimodal imaging. For detecting RPD, IR, FAF, and SD-OCT were more sensitive than color fundus photography. Although SD-OCT may be the most useful among the seven modalities, RPD detection should be performed using at least two imaging modalities for a more accurate diagnosis.

Key words: reticular pseudodrusen, age-related macular degeneration, scanning laser ophthalmoscopy, fundus autofluorescence, optical coherence tomography.

References

1. Mimoun G, Soubrane G, Coscas G. [Macular drusen]. J Fr Ophthalmol 1990;13:511–530.
2. Arnold JJ, Sarks SH, Killingsworth MC, et al. Reticular pseudodrusen. A risk factor in age-related maculopathy. Retina 1995;15:183–191.
3. Cohen SY, Dubois L, Tadayoni R, et al. Prevalence of reticular pseudodrusen in age-related macular degeneration with newly diagnosed choroidal neovascularization. Br J Ophthalmol 2007;91:354–359.
4. Sarks J, Arnold J, Ho I-V, et al. Evolution of reticular pseudodrusen. Br J Ophthalmol 2011;95:979–985.
5. Pumariega NM, Smith RT, Sohrab MA, et al. A prospective study of reticular macular disease. Ophthalmology 2011;118:1619–1625.
6. Schmitz-Valckenberg S, Alten F, Steinberg JS, et al. Reticular drusen associated with geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 2011;52:5009–5015.
7. Zweifel SA, Spaide RF, Curcio CA, et al. Reticular pseudodrusen are subretinal drusenoid deposits. Ophthalmology 2010;117:303–312.
8. Zweifel SA, Imaura Y, Spaide TC, et al. Prevalence and significance of subretinal drusenoid deposits (reticular pseudodrusen) in age-related macular degeneration. Ophthalmology 2010;117:1775–1781.
9. Lois N, Owens SL, Coco R, et al. Fundus autofluorescence in patients with age-related macular degeneration and high risk of visual loss. Am J Ophthalmol 2002;133:341–349.
10. Querques G, Querques L, Martinelli D, et al. Pathologic insights from integrated imaging of reticular pseudodrusen in age-related macular degeneration. Retina 2011;31:518–526.
11. Smith RT, Chan JK, Busuoic M, et al. Autofluorescence characteristics of early, atrophic, and high-risk fellow eyes in age-related macular degeneration. Invest Ophthalmol Vis Sci 2006;47:5495–5504.
12. Smith RT, Sohrab MA, Busuioc M, Barile G. Reticular macular disease. Am J Ophthalmol 2009;148:733–743.
13. Arnold JJ, Quaranta M, Soubrane G, et al. Indocyanine green angiography of drusen. Am J Ophthalmol 1997;124:344–356.
14. Spaide RF, Curcio CA. Drusen characterization with multimodal imaging. Retina 2010;30:1441–1454.
15. Keilhauner CN, Delori FC. Near-infrared autofluorescence imaging of the fundus: visualization of ocular melanin. Invest Ophthalmol Vis Sci 2006;47:3556–3564.
16. Kellner U, Kellner S, Weinitz S. Fundus autofluorescence (488NM) and near-infrared autofluorescence (787NM) visualize different retinal pigment epithelium alterations in patients with age-related macular degeneration. Retina 2010;30:6–15.

Prevalence and Genomic Association of Reticular Pseudodrusen in Age-Related Macular Degeneration

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• PURPOSE: To survey the prevalence of reticular pseudodrusen in late age-related macular degeneration (AMD) using multiple imaging methods, and to investigate the association between reticular pseudodrusen and polymorphisms in complement factor H (*CFH*) and age-related maculopathy susceptibility 2 (*ARMS2*) genes.

• DESIGN: Retrospective case series.

• METHODS: This study included 216 consecutive patients with late AMD (typical AMD, polypoidal choroidal vasculopathy [PCV], retinal angiomatous proliferation [RAP], or geographic atrophy). Eyes were assessed for reticular pseudodrusen using the blue channel of color fundus photography, infrared reflectance, fundus autofluorescence, and spectral-domain optical coherence tomography. The major AMD-associated single nucleotide polymorphisms (*CFH* Y402 rs1061170, *CFH* I62 V rs800292, and *ARMS2* A69S rs10490924) were genotyped.

• RESULTS: Forty-nine eyes of 30 patients had a reticular pattern in ≥2 imaging modalities and were diagnosed with reticular pseudodrusen. Of these, 16 had bilateral late AMD, whereas 32 of 186 patients without reticular pseudodrusen had bilateral late AMD ($P < .001$). The prevalence of reticular pseudodrusen was 83% in RAP, 50% in geographic atrophy, 9% in typical AMD, and 2% in PCV. The frequency of the T allele in *ARMS2* A69S in patients with and without reticular pseudodrusen was 78.6% and 59.9%, respectively ($P = .007$).

• CONCLUSIONS: The prevalence of reticular pseudodrusen was low in PCV cases. About 50% of patients with reticular pseudodrusen had bilateral late AMD. The connection of *ARMS2* risk allele and reticular pseudodrusen was confirmed in a Japanese population. (Am J Ophthalmol 2013;155:260–269. © 2013 by Elsevier Inc. All rights reserved.)

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RETICULAR DRUSEN, DESCRIBED IN THE WISCONSIN Grading System as one type of drusen that form ill-defined networks of broad interlacing ribbons, were first identified using blue-light fundus photography.[1,2] Arnold and associates described a yellowish interlacing network of oval-shaped or roundish lesions, termed reticular pseudodrusen, with a diameter of 125–250 μm that were seen in red-free fundus photography and infrared scanning-laser ophthalmoscopy (SLO).[3] Recently, reticular pseudodrusen have been recognized as an additional distinctive morphologic feature observed in age-related macular degeneration (AMD).[4] Furthermore, several reports have suggested that reticular pseudodrusen are associated with a high risk of progression to late AMD.[5–8] In the longitudinal Beaver Dam Eye Study, reticular pseudodrusen were found to confer a high risk of progression to late-stage AMD, with twice the risk compared with eyes with soft drusen.[8]

The development of new imaging methods, such as confocal SLO and spectral-domain optical coherence tomography (SD OCT), has led to improvements in diagnosing reticular pseudodrusen.[4,7–14] Previous reports showed that near-infrared reflectance (IR), fundus autofluorescence (FAF), and SD OCT were more useful than conventional fundus photography to detect reticular pseudodrusen and suggested that the assessment of reticular pseudodrusen should involve multiple imaging methods.[4,7-10,13,14]

Existing evidence suggests an association of AMD with polymorphisms in the complement factor H (*CFH*) gene and age-related maculopathy susceptibility 2 (*ARMS2*) gene.[15-24] Among the various polymorphisms, the Y402H and I62V variants in the *CFH* gene and the A69S variant in the *ARMS2* gene have been reported to show an association with AMD.[15-24] Recently an association between reticular pseudodrusen and polymorphisms in these genes has been reported.[8,25] Klein and associates showed that the prevalence of reticular pseudodrusen was higher in those homozygous (CC) or heterozygous (TC) for *CFH* Y402H than in those without this variant (TT).[8] On the other hand, Smith and associates demonstrated that *CFH* Y402H risk variant was significantly associated with the absence of reticular macular disease but enhanced risk for reticular macular disease was conferred by the *ARMS2* A69S risk allele.[25] Thus, to date, the association between reticular pseudodrusen and genomic

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background has not reached a consensus. In addition, little is known about the distribution of reticular pseudodrusen in each AMD subtype.

The purpose of this study was to survey the prevalence of reticular pseudodrusen in late AMD using multiple imaging methods and, moreover, to investigate the association of high-risk alleles in the *CFH* (Y402H, rs1061170 and I62V, rs800292) and *ARMS2* (A69S, rs10490924) genes with reticular pseudodrusen. Several terminologies have been used to describe this clinical feature.[2,3,9,14] In this report, we use the term "reticular pseudodrusen" according to the nomenclature by Arnold and associates.[3]

METHODS

WE RETROSPECTIVELY REVIEWED THE MEDICAL RECORDS OF 249 consecutive patients with newly diagnosed late AMD who first visited the Macular Service at Kyoto University Hospital between August 3, 2009 and July 21, 2011. Subjects included in this study were ≥50 years of age and had either typical AMD, polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP), or geographic atrophy. The diagnosis of PCV was based on the indocyanine green angiography (IA) showing a branching vascular network terminating in polypoidal swelling. The diagnosis of RAP was based on the criteria of Yannuzzi and associates[26] via fundus photography, fluorescein angiography (FA), IA, and SD OCT. Neovascular AMD other than PCV or RAP was defined as typical AMD. Geographic atrophy was defined using color fundus photography as a sharply delineated area (at least 175 μm in diameter) of hypopigmentation, depigmentation, or apparent absence of the retinal pigment epithelium (RPE) in which choroidal vessels were clearly visible. Eyes with other macular abnormalities (ie, pathologic myopia, idiopathic choroidal neovascularization, presumed ocular histoplasmosis, angioid streaks, other secondary choroidal neovascularization, central serous chorioretinopathy, epiretinal membrane, or retinal arterial macroaneurysm) were excluded from this study. All diagnoses were made by 3 retinal specialists (S.O., K.Y., and A.T.) who observed the images together and discussed each case; however, a fourth specialist (N.Y.) was consulted in case of a disagreement between the 3 initial reviewers. The fourth specialist made a decision in 13 of the 249 patients (5.2%). Patients were included only if at least 3 specialists agreed on the diagnosis.

All study investigations adhered to the tenets of the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board and the Ethics Committee of Kyoto University Graduate School of Medicine prior to the study. Written informed consent was obtained from all patients who were genotyped. Because this was a retrospective study, written informed consent for research participation was not obtained, but the nature of this study was explained on our website.

• MULTIMODAL IMAGING METHODS: All patients underwent a complete ophthalmologic examination, including measurement of best-corrected visual acuity, determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a noncontact lens, color fundus photography, SD OCT, IR, FAF, FA, and IA.

Color fundus photographs (field, 30-40 degrees) were obtained digitally using a Topcon TRC NW6S nonmydriatic retinal camera (Topcon, Tokyo, Japan) after medical dilation of the pupil (phenylephrine 0.5% and tropicamide 0.5%). To examine the blue channel of the color photography, ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) was used to display the individual color channels (red, green, and blue) of the obtained photographs. In ImageJ, the command path of Image > Color > Split Channels was used. Subsequently, the command path of Image > Adjust > Brightness/Contrast was used if needed. Adjustment was performed automatically using the ImageJ software before grading.

IR, FAF, FA, and IA images were acquired using a confocal SLO (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). The IR images were obtained using a light stimulus of 820 nm. The FAF images were obtained using an excitation light of 488 nm and a barrier filter beginning at 500 nm. The field of view was set to 30 × 30 degrees centered on the macula.

SD OCT was conducted using a Spectralis HRA+OCT (Heidelberg Engineering). First, horizontal and vertical line scans through the fovea center were obtained at a 30-degree angle, followed by serial horizontal scans with an examination field size ranging from 30 × 10 degrees to 30 × 25 degrees, depending on the case. At each location of interest on the retina, 50 SD OCT images were acquired and averaged to reduce speckle noise.

• DEFINITION OF THE RETICULAR PSEUDODRUSEN USING MULTIMODAL IMAGING: First, the quality of each image was evaluated by an experienced ophthalmologist (N.U.A.) and patients with adequate image quality in both eyes were included. Image quality was evaluated twice on all other days, and only images having an eligible quality during both evaluations were used. All these images were evaluated for the detection of reticular pseudodrusen by 2 independent experienced ophthalmologists (N.U.A. and S.O.). The evaluation of each image was performed referring to the corresponding images obtained from other imaging modalities. FA images were also referred to in order to distinguish reticular drusen from other lesions such as basal laminar drusen. In case of any discrepancy, a third experienced ophthalmologist (A.T.) was asked to arbitrate. In the current study, eyes diagnosed as having reticular pseudodrusen were those with reticular patterns in more than 2 of the following: the blue channel image

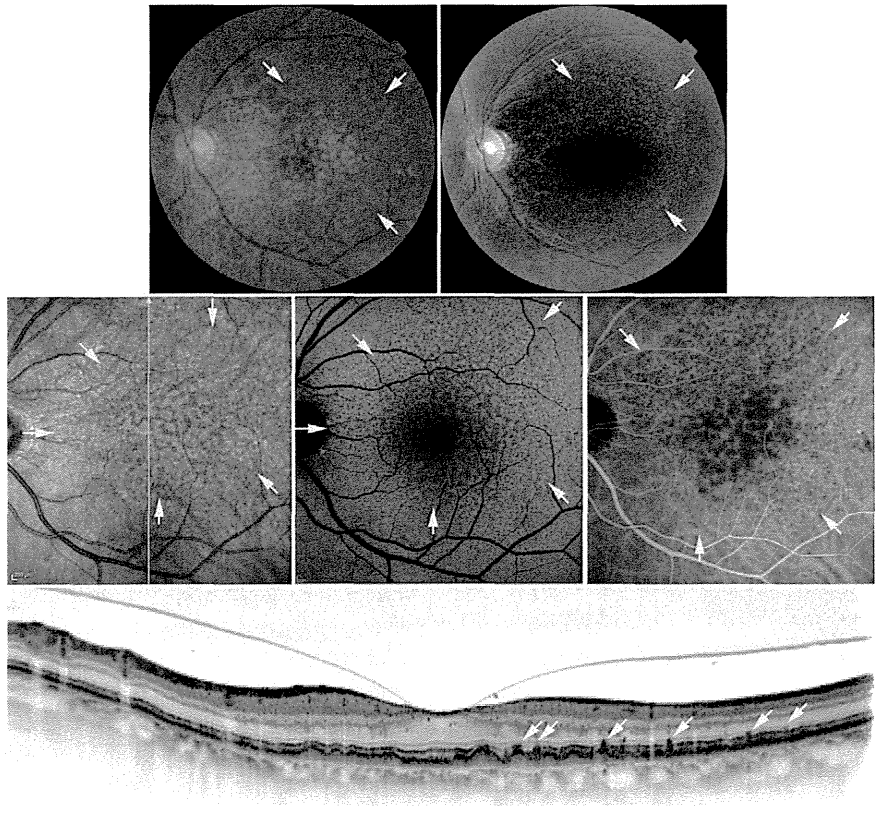

FIGURE 1. Reticular patterns in late age-related macular degeneration in multimodal imaging. (Top row, left) Color fundus photography. (Top row, right) Blue channel of contrast-enhanced color fundus photography. For color fundus or the corresponding blue channel of contrast-enhanced color fundus photography, reticular pattern is identified as light interlacing networks (arrows). (Second row, left) Infrared reflectance (IR). Reticular IR is identified as a grouping of hyporeflectant lesions against a background of mild hyperreflectance with analogous characteristics (arrows). (Second row, middle) Fundus autofluorescence (FAF). Reticular FAF is identified as a grouping of ill-defined, hypofluorescent lesions against a background of mildly elevated FAF (arrows). (Second row, right) Indocyanine green angiography (late phase). A pattern of hyporeflective dots is seen (arrows) corresponding to the reticular pattern in color fundus photography. (Bottom) Spectral-domain optical coherence tomography (SD OCT). Vertical line scan thorough the fovea in the direction of the green arrow in Second row, left shows reticular lesions identified as hyperreflective mounds or triangular lesions above the retinal pigment epithelium (arrows).

of color fundus photography, IR, FAF, or SD OCT. For the blue channel of contrast-enhanced color fundus photography, a reticular pattern was identified as light interlacing networks that were 125-250 μm wide (Figure 1).[3] Reticular autofluorescence was defined as a group of ill-defined, hypofluorescent lesions against a background of mildly elevated AF (Figure 1).[11,13] Reticular IR was defined as a group of hyporeflectant lesions against a background of mild hyperreflectance with analogous characteristics (Figure 1).[14] SD OCT reticular lesions were defined as ≥5 hyperreflective mounds or triangular lesions above the RPE in ≥1 B-scan (Figure 1).[10]

• GENOTYPING: Genomic DNA was prepared from leukocytes of peripheral blood with a DNA extraction kit (QuickGene-610L; Fujifilm, Tokyo, Japan). Of the 216 patients who met the inclusion criteria, genomic data from 11 patients were not available because of the following reasons: (1) consensus of blood extraction was not achieved; (2) genotyping was not possible because of the preservation state. Thus, analyses for genomic data were limited to 205 patients. We genotyped the major AMD-associated single nucleotide polymorphism (SNP), *CFH* Y402 rs1061170, I62 V rs800292, and *ARMS2* A69S rs10490924. The SNPs were genotyped using TaqMan SNP assays with the ABI PRISM 7700 system (Applied Biosystems Inc, Foster City, California, USA), according to the manufacturer's instructions.

• STATISTICAL ANALYSIS: Statistical analysis was performed using SPSS 17 software (SPSS Inc, Chicago, Illinois, USA). All values are presented as a mean ± standard deviation (SD). For statistical analysis, visual acuity measured using a Landolt chart was converted to the logarithm of the minimal angle of resolution (logMAR). Mann-Whitney *U* tests were used to compare data from 2 groups

TABLE 1. Characteristics of Patients With Late Age-Related Macular Degeneration in This Study

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients (%)	97 (44.9)	87 (40.2)	12 (5.6)	12 (5.6)	8 (3.7)	216 (100)
Sex, n (%)						
Men	76 (78)	70 (80)	4 (33)	7 (58)	4 (50)	161 (75)
Women	21 (22)	17 (20)	8 (67)	5 (42)	4 (50)	55 (25)
No. of affected eyes (%)						
Two	17 (18)	9 (10)	5 (42)	9 (75)	8	48 (22)
One	80 (82)	78 (90)	7 (58)	3 (25)	–	168 (78)
Age (mean ± SD)	74.8 ± 8.3	71.5 ± 8.4	81.3 ± 8.2	72.3 ± 9.3	82.3 ± 2.9	73.9 ± 8.7

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (4 typical AMD and PCV, 1 typical AMD and RAP, 1 typical AMD and geographic atrophy, 1 geographic atrophy and RAP, and 1 geographic atrophy and PCV).

in which normal distributions were not verified. To compare ratios between the 2 groups, χ^2 tests were used. $P < .05$ was considered statistically significant.

RESULTS

IN THIS STUDY, DATA OF 249 CONSECUTIVE PATIENTS WITH late AMD were retrospectively reviewed; however, 5 patients with an eye with phthisis bulbi and 28 patients with poor image quality were excluded. (The intraobserver agreement for grading of image quality was 94.6%.) Thus, 216 patients were included in this study. All patients were Japanese. The patients comprised 161 men and 55 women, aged 51-92 years (mean ± SD, 73.9 ± 8.7). Among them, 97 patients (44.9%) had typical AMD, 87 (40.2%) had PCV, 12 (5.6%) had RAP, and 12 (5.6%) had geographic atrophy. Eight patients had a different type of late AMD in both eyes that was defined as "combined." (Four patients had typical AMD in 1 eye and PCV in the other eye. The other combinations were typical AMD and geographic atrophy, typical AMD and RAP, geographic atrophy and PCV, and geographic atrophy and RAP. The visual acuity of these patients ranged from 20/2000 to 20/12 (mean logMAR = 0.33 ± 0.52). Spherical equivalent refractive error ranged from -5.50 diopters (D) to +4.50 D in the eyes with late AMD, and ranged from -18.375 D to +3.875 D in the fellow eyes without late AMD (4 eyes with high myopia [<-6 D] were included in the fellow eyes). Sixty-eight eyes had pseudophakia. The characteristics of the participants are summarized in Table 1.

Using color fundus photography, IR, FAF, or SD OCT, it was determined that out of 432 eyes, 30 eyes (6.9%), 65 eyes (15.0%), 45 eyes (10.4%), and 47 eyes (10.9%), respectively, had a reticular pattern. (Inter- and intraobserver agreements for grading for the detection of reticular pseudodrusen are shown in Table 2.) Furthermore, 49 eyes (11.3%) of 30 patients had a reticular pattern according to ≥2 imaging modalities, and were defined as having reticular pseudodrusen. Reticular pseudodrusen was confirmed bilaterally in 19 of these 30 patients (63.3%) and unilaterally in 11 patients (36.7%). In all 11 patients with unilateral reticular pseudodrusen, the other eye had neovascular AMD (4 were RAP, 6 were typical AMD, and 1 was PCV). In 38 of 49 eyes (77.6%) with reticular pseudodrusen, a pattern of hyporeflective dots was detected in the middle- and late-phase IA corresponding to the reticular pattern detected in IR and FAF (Figure 1).

The characteristics of patients with reticular pseudodrusen (30 patients [13.9%]) and patients without reticular pseudodrusen (186 patients) are summarized in Tables 3 and 4, respectively. Of the 30 patients with reticular pseudodrusen, 19 (63.3%) were women, whereas only 36 of 186 patients without reticular pseudodrusen (19.4%) were women ($P < .001$, χ^2 test). The mean age of the patients with reticular pseudodrusen was 80.6 ± 6.8 years (range, 65-92 years), which was significantly higher than that of patients without reticular pseudodrusen (72.8 ± 8.5 years; range, 51-92 years; $P < .001$, Mann-Whitney test). In addition, 16 of 30 patients with reticular pseudodrusen (53.3%) had bilateral late AMD, whereas only 32 of 186 patients without reticular pseudodrusen (17.2%) had bilateral late AMD ($P < .001$, χ^2 test). In patients over 70 years old, 15 of 28 patients with reticular pseudodrusen (53.6%) had bilateral late AMD, whereas 26 of 118 patients without reticular pseudodrusen (22.0%) had bilateral late AMD ($P = .001$, χ^2 test).

The prevalence rate of reticular pseudodrusen was different according to the disease type of late AMD: 10 of 12 patients with RAP (83.3%), 6 of 12 patients with geographic atrophy (50.0%), 9 of 97 patients with typical AMD (9.2%), 2 of 87 patients with PCV (2.2%), and 3 of 8 patients with combined subtype (Table 5). In patients over 70 years old, the prevalence of reticular pseudodrusen was 10 of 11 patients with RAP (90.9%), 5 of 7 patients

TABLE 2. Intra- and Interobserver Agreements for Grading of the Detection of Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Blue Channel[a]	IR	FAF	SD OCT
Intraobserver agreements				
Accordance rate (%)	96.9	94.6	95.3	98.3
Kappa coefficient (95% confidence interval)	0.72 (0.57-0.86)	0.78 (0.69-0.86)	0.74 (0.63-0.85)	0.91 (0.85-0.98)
Interobserver agreements				
Accordance rate (%)	94.7	91.4	91.2	97.9
Kappa coefficient (95% confidence interval)	0.62 (0.47-0.76)	0.64 (0.54-0.75)	0.61 (0.50-0.72)	0.89 (0.81-0.96)

FAF = fundus autofluorescence; IR = near-infrared reflectance; SD OCT = spectral-domain optical coherence tomography.
[a]Blue channel of color fundus photography.

TABLE 3. Characteristics of Patients With Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients	9	2	10	6	3	30
Sex, n (%)						
Men	5 (56)	0	3 (30)	2 (33)	1 (33)	11 (37)
Women	4 (44)	2 (100)	7 (70)	4 (67)	2 (67)	19 (63)
No. of eyes with AMD (%)						
Two	3 (33)	0 (0)	4 (40)	6 (100)	3	16 (53)
One	6 (67)	2 (100)	6 (60)	0 (0)	—	14 (47)
Age (mean ± SD)	79.1 ± 6.6	82.0 ± 4.2	82.4 ± 7.7	78.3 ± 8.0	83.0 ± 2.6	80.6 ± 6.8

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.
[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (1 RAP and GA, 1 AMD and RAP, and 1 AMD and PCV).

with geographic atrophy (71.4%), 8 of 67 patients with typical AMD (11.9%), and 2 of 53 patients with PCV (3.8%) (Supplemental Table, available at AJO.com).

In 28 patients with reticular pseudodrusen and 177 patients without reticular pseudodrusen, the frequency of the minor allele in *CFH* I62 V polymorphism was 21.4% and 23.0%, respectively (Table 6). Upon analyzing the genotype, we determined the G allele did not contribute to reticular pseudodrusen ($P = .865$). The frequency of the C allele in *CFH* Y402H was 14.3% and 16.7%, respectively, in the patients with and without reticular pseudodrusen (Table 6). The 2×2 table from the allele χ^2 test revealed no C allele contribution to reticular pseudodrusen ($P = .845$).

In contrast, the A69S polymorphism in the *ARMS2* gene apparently contributed to reticular pseudodrusen (Table 6). The frequency of homozygosity for the at-risk genotype (TT) of A69S was 60.7% and 38.4%, respectively, in patients with and without reticular pseudodrusen. Furthermore, the frequency of the T allele in A69S was 78.6% and 59.9%, respectively. When examined with a 2×2 table from the allele χ^2 test, we found the T allele contributed significantly to reticular pseudodrusen ($P = .007$).

DISCUSSION

RETICULAR PSEUDODRUSEN HAS TRADITIONALLY BEEN identified with blue-light fundus photography. However, with the development of various imaging modalities, recent studies have suggested that additional methods such as FAF, IR imaging, and SD OCT would facilitate the identification of reticular pseudodrusen.[4,5,7,9,10,13,14] In all these cited studies, multiple imaging modalities were used to detect reticular pseudodrusen, with diagnosis based on the least positive modality. However, the reticular pattern is sometimes subtle and difficult to distinguish from other alterations (soft/hard drusen) when using only 1 imaging modality (Figure 2). Therefore, we used 4 imaging methods. Reticular pseudodrusen-affected eyes were defined as those with reticular patterns discovered with the use of >2 imaging modalities. Among these 4 modalities, IR showed the highest sensitivity for detecting reticular pseudodrusen, consistent with previous studies.[9,14]

Previous reports suggest that IA is also useful for detecting reticular pseudodrusen.[14,27,28] Here, we reviewed IA images from patients with reticular pseudodrusen. In many eyes, hyporeflective dots were detected in the

TABLE 4. Characteristics of Patients Without Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients	88	85	2	6	5	186
Sex, n (%)						
Men	71 (81)	70 (82)	1 (50)	5 (83)	3 (60)	150 (81)
Women	17 (19)	15 (18)	1 (50)	1 (17)	2 (40)	36 (19)
No. of eyes with AMD (%)						
Two	14 (16)	9 (11)	1 (50)	3 (50)	5 (100)	32 (17)
One	74 (84)	76 (89)	1 (50)	3 (50)	—	154 (83)
Age (mean ± SD)	74.2 ± 8.3	71.3 ± 8.4	76.0 ± 11.3	66.2 ± 6.2	81.8 ± 3.3	72.8 ± 8.5

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (1 RAP and geographic atrophy, 1 AMD and RAP, and 1 AMD and PCV).

TABLE 5. Prevalence of Reticular Pseudodrusen in Each Disease Type of Late Age-Related Macular Degeneration

	No. of Patients	No. of Patients With Reticular Pseudodrusen	Prevalence (%)
Typical AMD	97	9	9.2
PCV	87	2	2.2
RAP	12	10	83.3
Geographic atrophy	12	6	50.0
Combined[a]	8	3	37.5

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (4 typical AMD and PCV, 1 typical AMD and RAP, 1 typical AMD and geographic atrophy, 1 geographic atrophy and RAP, and 1 geographic atrophy and PCV).

middle and late phases of reticular pseudodrusen development. These dots correspond to the reticular pattern detected in IR and FAF. Smith and associates reported that IA detected reticular pseudodrusen with 100% sensitivity, although the sample size was small.[14] Our findings suggest that IA is a useful method to detect reticular pseudodrusen.

In our study, the proportion of women was higher in the reticular pseudodrusen group than in the group without reticular pseudodrusen. Patients with reticular pseudodrusen were older than those without. These results are consistent with previous reports indicating that women and older patients are more likely to have reticular pseudodrusen.[3,4,10,14] In addition, many reticular pseudodrusen patients in our study had bilateral late AMD. Arnold and associates reported that CNV was found in 66 of 100 patients with reticular pseudodrusen; bilateral CNV was found in 24 patients.[3] Pumariega and associates showed that reticular pseudodrusen was associated with progression to late AMD in the fellow eye.[6] Thus, ophthalmologists should be aware that patients with reticular pseudodrusen have the risk of bilateral late AMD and should conduct follow-ups on these patients.

The prevalence of reticular pseudodrusen has been described in several reports. A population-based study revealed that the overall prevalence was 0.7% in the general population. The associated 15-year incidence increased from 0.4% to 6.6% with age, which is similar to the trend observed for AMD.[8] In AMD patients, the prevalence of reticular pseudodrusen ranged from 9% to 36%.[3,4,10,13] In the current study, reticular pseudodrusen was detected in 14% of patients with late AMD. This small number may be explained by the following reasons: the diagnosis of reticular pseudodrusen was based on reticular patterns as observed in ≥2 imaging modalities; our cohort included many patients with PCV, in whom reticular pseudodrusen was rarely detected; our subjects were younger (mean age, 73.9 years) than the subjects studied by Cohen and associates (mean age, 79.5 years) [4]; and our study had relatively fewer women than those in previous reports, although the sex distribution was similar to that of other Japanese AMD studies.[29] Considering that women are more likely than men to have reticular pseudodrusen, sex distribution may be one of the reasons for low prevalence of reticular pseudodrusen. Reticular pseudodrusen may fade with post-CNV development.[14] Smith and associates included only the fellow eyes of patients with unilateral CNV.[13] The current study included patients with bilateral CNV; some of these patients may have had reticular pseudodrusen before CNV development. We speculate that there may be ethnic differences in the reticular pseudodrusen prevalence between Japanese and white populations, similar to the varying prevalence of soft drusen among AMD patients.[30-32] A recent Korean study indicated that ethnic differences may be associated with certain clinical features.[33]

TABLE 6. Distribution of Genotype of *ARMS2* A69S, *CFH* I62 V, and *CFH* Y402 in Patients With vs Without Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Reticular Pseudodrusen (+) (n=28)					Reticular Pseudodrusen (-) (n=177)					
	Genotype, n (%)			Allele, n (%)		Genotype, n (%)			Allele, n (%)		P^a
ARMS2 A69S	GG	GT	TT	G	T	GG	GT	TT	G	T	
	1 (3.6)	10 (35.7)	17 (60.7)	12 (21.4)	44 (78.6)	33 (18.7)	76 (42.9)	68 (38.4)	142 (40.1)	212 (59.9)	.007
CFH I62 V	AA	AG	GG	A	G	AA	AG	GG	A	G	
	4 (14.3)	4 (14.3)	20 (71.4)	12 (21.4)	44 (78.6)	16 (9.1)	49 (27.8)	111 (63.1)	81 (23.0)	271 (77.0)	.865
CFH Y402	CC	CT	TT	C	T	CC	CT	TT	C	T	
	1 (3.6)	6 (21.4)	21 (75.0)	8 (14.3)	48 (85.7)	7 (4.0)	45 (25.4)	125 (70.6)	59 (16.7)	295 (83.3)	.845

[a]*P* value with 2 × 2 table of allele χ^2 test for its exact counterpart.

In the current study, reticular pseudodrusen prevalence was high in eyes with RAP or geographic atrophy, consistent with previous reports.[5,6] Therefore, reticular pseudodrusen may represent a hallmark or adverse effect associated with the pathology of RAP and geographic atrophy. In contrast, reticular pseudodrusen was rarely found in PCV patients (2%). To our knowledge, this is the first report on reticular pseudodrusen prevalence in PCV patients.

Literature suggests an association between AMD and polymorphisms in the *CFH* gene.[15-19,24] The Y402H and I62 V variants, in particular, have been specifically reported to be associated with AMD.[15-19,24] In a Japanese cohort, we have previously shown that both *CFH* Y402H and I62 V are associated with AMD.[19] The Beaver Dam Eye Study showed a higher prevalence of reticular pseudodrusen in participants carrying the Y402H mutation of the C allele, which is associated with an increased risk of AMD.[8] In contrast, Smith and associates reported that this *CFH* variant was significantly associated with absence of reticular pseudodrusen.[25] We found no association between reticular pseudodrusen and *CFH* Y402H in patients with late AMD. As the frequency of the Y402H C allele is very low in the Asian population,[34-36] it may be difficult to show a significant difference. In contrast, I62 V is more suitable for an association study focusing on the *CFH* gene in Japanese people, because its minor allele frequency is approximately 40%.[19,24] However, in the current study, we found no association between I62 V and reticular pseudodrusen.

The A69S variant of the *ARMS2* gene is also associated with AMD; this association was reported in white and Asian subjects.[19-23,37-42] Smith and associates reported that the *ARMS2* A69S allele increases the risk for reticular pseudodrusen.[25] In the current study, the T-allele frequencies of the *ARMS2* A69S were higher in reticular pseudodrusen patients, which suggests that the A69S polymorphism contributed to reticular pseudodrusen development. Although we reported higher T-allele frequencies for *ARMS2* A69S than Smith and associates,[25] the risk-allele frequency in patients without reticular pseudodrusen was similar to that reported in previous studies on Japanese patients.[43,44] The finding that both the *ARMS2* risk allele and reticular pseudodrusen apparently lead to late AMD may suggest a common mechanism. Although the function of *ARMS2* is unknown, Fritsche and associates showed that *ARMS2* was expressed in the ellipsoid region of the photoreceptor inner segments.[39] Despite the controversial localization of reticular pseudodrusen, several researchers have shown that reticular pseudodrusen location corresponds to that of abnormal material above the RPE.[9,45] Thus, *ARMS2* expression may colocalize with reticular pseudodrusen, raising the possibility that *ARMS2* may play a role in the formation of reticular pseudodrusen. Meanwhile, other reports suggest that the reticular pattern is related to impaired choroidal filling, as observed in our subjects, and may involve the RPE, choriocapillaris, and inner choroid.[3,14,46] Querques and associates proposed that derangement of the RPE attributable to underlying atrophy and fibrosis of the choroid might lead to the accumulation of photoreceptor outer segments above the RPE.[28] Kertvely and associates showed that *ARMS2* gene expression localized primarily to the intercapillary area of the choroid.[47] Further investigations are required on the histology of reticular pseudodrusen and the locations and functions of *ARMS2* during the course of AMD.

Our study had several limitations. First, this study was retrospective, and the imaging protocol was not standardized. Second, the sample size was relatively small compared with that used in other prevalence and genetic studies, and we included several disease types. Third, a single grader subjectively evaluated image quality. When the same researcher evaluated it again, intra-observer agreement was high. Fourth, our results show that the prevalence of reticular pseudodrusen was high in patients with RAP or geographic atrophy, but the small sample size of these patients prevented us from establishing this trend. Finally, the techniques used here may have failed to identify reticular pseudodrusen in eyes in which reticular pseudodrusen was present only on the nasal side of the optic disc.[13] Furthermore, some patients with