

Prevalence and Risk Factors for Age-Related Macular Degeneration in an Adult Japanese Population

The Funagata Study

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Objective: To describe the prevalence and risk factors for age-related macular degeneration (AMD) in a Japanese population and to compare these with data from a white population.

Design: Population-based cross-sectional epidemiologic study.

Participants: A population-based sample of Japanese persons 35 years or older from Funagata, Japan.

Methods: The Funagata study is a population-based study of 1758 (43% of eligible) Japanese persons 35 years or older from Funagata, Japan. In 2000 to 2002, 1625 (92.4%) participants had a nonmydriatic fundus photograph of one eye with sufficient quality for grading of AMD lesions, using the Wisconsin protocol. Age-standardized prevalence rates compared with the Blue Mountains Eye Study (BMES) population, odds ratios (ORs), and 95% confidence intervals (CIs) were calculated. Risk factors were assessed by logistic regression.

Main Outcome Measures: Early and late AMD.

Results: Of 1625 participants, early AMD and late AMD were present in 3.5% and 0.5%, respectively. Age-standardized early AMD prevalence in right eyes was 4.1%, similar to the corresponding prevalence of 4.4% in the BMES. For men, age-standardized prevalences of late AMD in right eyes were 1.1% and 1.2% in the BMES; for women, the corresponding prevalences were 0.3% and 2.1%, respectively. Increasing age (per 10 years; gender-adjusted OR, 2.27; 95% CI, 1.10–4.67) and current cigarette smoking (age- and gender-adjusted OR, 5.03; 95% CI, 1.00–25.47) were associated with late AMD.

Conclusions: In this Japanese population, prevalence of early AMD was similar to that for whites in the BMES. Although the late AMD prevalence was lower in Japanese women, in Japanese men it was similar to that in whites. This could have resulted from the substantially high proportion of Japanese men who are smokers. Cigarette smoking and increasing age were the 2 principal factors found associated with late AMD. *Ophthalmology* 2008;115:1376–1381 © 2008 by the American Academy of Ophthalmology.



In the last 2 decades, several population-based studies have reported on the prevalence of age-related macular degeneration (AMD) in different racial/ethnic groups.^{1–6} These

studies suggest that AMD is more common in whites and less common in blacks.^{7–9}

It has long been hypothesized that Asians in general and Japanese people in particular may have lower rates of AMD.¹⁰ However, there are few data in Japanese popula-

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tions to support this concept. In the Hisayama Study, a relatively low prevalence of late AMD (0.87%) was reported in a population-based sample of Japanese ≥ 50 -year-olds⁷ as compared with other studies of white populations, such as the Blue Mountains Eye Study (BMES). The prevalence of early AMD in the Hisayama Study (12.7%), however, was reportedly higher than that in the BMES (7.2%).² Different definitions of early AMD were used in the Hisayama Study⁷ and BMES²; that comparison is limited, as different definitions of early AMD have been shown to result in varied prevalence within the same population.⁶

Cigarette smoking is a major risk factor for AMD in white populations.¹¹ However, this association has not been consistently demonstrated in either Chinese¹² or Japanese^{7,13} populations. Given the high prevalence of smoking in Asia and in Japan,^{14,15} further studies evaluating the relationship of cigarette smoking and AMD in Japan are clearly needed.

We had 2 purposes for conducting this study. First, we aimed to describe the prevalence of AMD in a Japanese population (the Funagata Study), in which the assessment of AMD was performed at the image-reading center of the Centre for Vision Research, University of Sydney, using definitions identical to those used in the BMES. We sought to compare the prevalence of early and late AMD between Japanese and the white BMES population after age standardization. Second, we aimed to examine risk factors associated with early or late AMD in Japanese adults.

Materials and Methods

Study Population

The Funagata Study is a population-based study among adult Japanese.^{16–18} The study population and methods are described in detail elsewhere.¹⁶ In brief, between June 2000 and June 2002, 4160 residents of Funagata, Japan 35 years or older were identified. After excluding 484 with severe disabilities such as hemiparesis after stroke, severe mental diseases, or dementia and individuals receiving treatment for diabetes, 3676 were identified as eligible subjects. Of those, 1961 (53.3% of eligible) were examined; more eligible women participated in this study than eligible men (57.6% vs. 51.5%, $P = 0.002$). The age-specific participation rate was lowest (24%) in the 35- to 40-year age group, increased to 63% in the 65 to 70 and 70 to 75 age groups, and then decreased to 50% in the ≥ 80 age group. Of the 3676 eligible persons, 1758 (47.8%) had a detailed ophthalmic examination and were included. The study was conducted according to the recommendations of the Declaration of Helsinki and was approved by institutional review boards at Yamagata University Faculty of Medicine.

Fundus Photography

Nonstereoscopic fundus photographs of one eye (generally the right eye) were obtained using a 45° nonmydriatic fundus camera (either CR5-NM45 [Canon Inc., Tokyo, Japan] or TRC-NW [Topcon Inc., Tokyo, Japan]) after sitting in a darkened room for around 5 minutes without using pharmacological dilating agents; those images were recorded on 35-mm slide film. Images were centered on the optic disc and macula. If the fundus photography of the right eye was not possible because of media opacity or other reasons, photographs of the left eye were taken.

Grading of Fundus Photographs for Age-Related Macular Degeneration

One thousand six hundred twenty-five (92.4%) participants had a nonmydriatic fundus photograph of one eye with sufficient quality for grading of AMD lesions. All fundus photographs were evaluated at the Centre for Vision Research by the team that conducted the BMES (Principal Investigators: PM, JJW). Details of the AMD photograph grading followed protocols used for the BMES, as described elsewhere.^{2,16} In brief, a trained grader (BT) assessed photographs for AMD signs in masked fashion, following the modified Wisconsin Age-Related Maculopathy Grading System¹⁹ protocol used in the BMES,² with adjudication provided by a senior researcher (JJW) and retinal specialist (PM). We used the International Age-Related Maculopathy Epidemiological Study Group nomenclature to describe AMD lesions.²⁰

Definition of Lesions in Early Age-Related Macular Degeneration

Drusen type was classified based on the size and sharpness of the edges; drusen were classified as hard or soft; soft drusen were then divided into distinct and indistinct types by the appearance of their borders.^{2,20} The grading of maximal lesion size within the macula was estimated using grading circles measuring 63 μm , 125 μm , 175 μm , 250 μm , and 500 μm . Retinal pigmentary abnormalities were graded to hypopigmentation and hyperpigmentation; hypopigmentation was defined as an area of retinal pigment epithelial depigmentation without visible choroidal vessels, and hyperpigmentation as increased pigment beneath the retina associated with drusen.^{2,20} Early AMD was defined using the BMES definition as either soft indistinct or reticular drusen or soft distinct drusen plus retinal pigment epithelium (RPE) abnormalities.²

Definition of Lesions in Late Age-Related Macular Degeneration

Neovascular AMD lesions included the presence of RPE detachment, with or without neurosensory detachment; subretinal or sub-RPE hemorrhages; or epiretinal, intraretinal, subretinal, or sub-RPE scar tissue.^{2,20} Subretinal hemorrhages or hard exudates within the macular area related to the above lesions and not related to other retinal vascular disease were also included as neovascular AMD.^{2,20} Geographic atrophy was defined by presence of visible choroidal vessels and a discrete border and needed to be of an area at least equal to a circle 175 μm in diameter.^{2,20} Late AMD was defined as either neovascular AMD or geographic atrophy.

Assessment of Risk Factors

Details of systemic assessment are provided elsewhere.¹⁶ In summary, blood pressure (BP) was measured after participants were seated comfortably for at least 5 minutes. A single measure of systolic and diastolic BPs was used. Hypertension was defined using the 2003 World Health Organization guidelines²¹; persons with hypertension stages I to III (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg) or a previous diagnosis of hypertension were defined as having hypertension. Diabetes and glucose tolerance status were defined using the results of a 75-g oral glucose tolerance test, following World Health Organization guidelines.²² Prediabetes was defined as either impaired glucose tolerance or impaired fasting glucose. Total cholesterol and high-density lipoprotein cholesterol were also measured. Self-reported smoking status was assessed. Body mass index was calculated as weight (kilograms) divided by the square of height (meters).

Data Analysis

Age- and gender-specific prevalences of individual AMD lesions, plus early AMD and late AMD, were assessed. Analysis of variance and analysis of covariance or the chi-square test was used to compare demographic characteristics. Direct age standardization of our study population to the BMES population was conducted to compare early and late AMD prevalences using data from right eyes for the 2 populations.² Logistic regression models were used to determine the odds of early or late AMD by unit change for a risk category versus a reference category. Possible risk factors included in the analyses were age, gender, systolic and diastolic BPs, hypertension (present vs. absent), body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking status (current smoker vs. never or past smoker), fasting plasma glucose, 2-hour postload glucose, and diabetes status (prediabetes or diabetes vs. nondiabetes). SPSS software (version 13.0, SPSS Inc., Chicago, IL) was used for statistical analyses.

Results

Participants with adequate photographs were significantly younger (60.2 vs. 69.4 years, $P < 0.0001$) than participants with poorer photographs. There were no differences in other risk factors between those included in the analyses and those excluded from them (data not shown). The age distribution of the participants is shown in Figure 1 (available at <http://aaojournal.org>).

The prevalence of early AMD was 3.5% among all participants 35 years and older or 4.3% among those 50 and over. Corresponding prevalences of late AMD were 0.5% and 0.6%, respectively, among participants 35 and older and 50 and older. Participant characteristics by the presence or absence of early and late AMD are shown in Table 1. Persons with early AMD were significantly older than those without early AMD. Persons with late AMD were significantly older and more likely to be current cigarette smokers than those without late AMD. There were more men with late AMD than women, but there was no statistically significant gender difference after adjusting for age.

Table 2 shows the prevalence of specific AMD lesions by age and gender. Overall prevalences of distinct drusen, indistinct drusen, and large drusen ($>125 \mu\text{m}$) were 15.1%, 1.7%, and 15.6%, respectively; there was an age-related trend ($P < 0.0001$). Men were more likely to have distinct drusen and large drusen than women ($P_s = 0.02$ and 0.03 , respectively). Prevalences of hyper-

pigmentation and hypopigmentation were 4.0% and 4.2%, respectively, but these lesions showed no age-related trend ($P_s = 0.83$ and 0.76 , respectively). Pigmentary abnormalities were more frequent in men than in women; this difference was statistically significant for hypopigmentation (5.6% vs. 2.7%, $P = 0.01$) but not for hyperpigmentation (5.1% vs. 3.5%, $P = 0.16$). Although late AMD and its component lesions, neovascular AMD and geographic atrophy, were more prevalent in men (0.8%, 0.6%, and 0.4% in men, vs. 0.2%, 0.1%, and 0.1% in women), these gender differences were not statistically significant ($P_s = 0.08$, 0.24 , and 0.45 , respectively).

We compared age-standardized prevalences of early and late AMD in the right eyes of our population with the corresponding prevalences in the right eyes of BMES participants (Table 3 [available at <http://aaojournal.org>]). The age-standardized prevalence of early AMD in our study (4.2%) was similar to that in the BMES (4.4%). For men, the age-standardized prevalence of late AMD in right eyes (1.1%) was also similar to that in the BMES (1.2%), but the corresponding late AMD prevalence in women differed between the 2 studies (0.3% in this study and 2.1% in the BMES).

Table 4 (available at <http://aaojournal.org>) shows associations of risk factors with AMD. Older age was associated with increased odds of both early and late AMD after adjusting for gender (early AMD: odds ratio [OR], 1.75; 95% confidence interval [CI], 1.36–2.25) (late AMD: OR, 2.27; 95% CI, 1.10–4.67), per 10-year increase in age. Current smoking was associated with late AMD after adjusting for age and gender (OR, 5.03; 95% CI, 1.00–25.47). This association was stronger in men (OR, 6.19; 95% CI, 1.08–35.5). There was no significant association between current smoking and early AMD. Although 2-hour postload glucose was associated with early AMD, this association was reduced after adjusting for age and gender. None of the other factors assessed was found to be associated with either early or late AMD.

Discussion

In this adult Japanese population, prevalences of early and late AMD, defined from right eyes only, were 3.5% and 0.5%, respectively, among those 35 years or older. Our study sample was weighted with relatively older persons, which may have led to an overestimation of AMD prevalence. After direct age standardization of our study sample to the BMES population, the prevalence of early AMD in

Table 1. Demographic Characteristics by Presence or Absence of Early and Late Age-Related Macular Degeneration (AMD) in the Funagata Study, Japan 2000–2002

Characteristic	No AMD (n = 1559)	Early AMD (n = 58)	Late AMD (n = 8)
Age (yrs)	60.1±12.2	67.5±9.1	70.6±6.8
Female gender (%)	56.0	56.9	25.0
Systolic blood pressure (mmHg)	127.4±16.8	130.6±17.5	129.3±11.6
Diastolic blood pressure (mmHg)	75.9±10.0	77.4±10.4	79.3±10.0
Hypertension (%)	35.4	43.1	50.0
Current smoker (%)	18.0	17.2	50.0
Body mass index (kg/m ²)	23.8±3.5	23.2±3.1	22.4±2.7
Total cholesterol (mg/dl)	201.7±33.8	198.5±27.5	191.6±35.3
High-density lipoprotein cholesterol (mg/dl)	58.5±14.5	60.8±14.1	55.6±10.3
Fasting plasma glucose (mg/dl)	98.5±27.0	97.9±23.5	93.4±3.7
2-hr postload glucose (mg/dl)	122.4±46.2	135.4±67.3	132.0±31.1
Prediabetes (%)	18.9	17.2	37.5
Diabetes (%)	9.9	12.7	0.0

Table 2. Prevalence of Age-Related Macular Degeneration (AMD) Lesions (Right Eye) in the Funagata Study, Japan 2000–2002

	Age Groups (yrs)	No. at Risk	Drusen (%)		Pigmentary Abnormality (%)		Early AMD (%)	Late AMD (%)
			Soft Distinct Drusen	Soft Indistinct Drusen	Hyperpigmentation	Hypopigmentation		
Men	35–54	259	5.0	0.4	4.6	5.8	0.0	0.0
	55–64	143	17.5	1.4	4.2	5.6	0.0	0.0
	65–74	211	24.6	2.8	6.6	6.6	1.4	1.4
	≥75	99	35.4	2.0	4.0	3.0	3.0	3.0
	Total	712	17.6	1.5	5.1	5.6	0.8	0.8
P value for age trend			<0.001	0.10	0.75	0.53	0.04	0.03
Women	35–54	329	4.0	0.3	3.0	2.1	0.0	0.0
	55–64	205	10.2	1.0	3.4	2.4	0.5	0.5
	65–74	278	22.3	3.2	4.3	2.9	0.4	0.4
	≥75	101	24.8	5.0	3.0	5.0	0.0	0.0
	Total	913	13.3	1.9	3.5	2.7	0.2	0.2
P value for age trend			<0.001	<0.001	0.89	0.29	<0.001	0.57
P values between genders			0.02	0.77	0.16	0.01	0.89	0.08
Total	35–54	588	4.4	0.3	3.7	3.7	0.0	0.0
	55–64	348	13.2	1.2	3.7	3.7	0.3	0.3
	65–74	489	23.3	3.1	5.3	4.5	0.8	0.8
	≥75	200	30.0	3.5	3.5	4.0	1.5	1.5
	Total	1625	15.1	1.7	4.2	4.0	0.5	0.5
P value for age trend			<0.001	<0.001	0.83	0.76	<0.001	0.02

our Japanese participants (4.1%) was quite similar to that in the right eyes of BMES participants (4.4%). For men, the age-standardized prevalence of late AMD in our sample (1.1%) was also comparable to that in the BMES (1.2%), but for women, it was lower than in the BMES (0.3% and 2.1%, respectively). The overall late AMD prevalence of 0.7% in this Japanese sample is lower than that in the BMES (1.8%). We also documented 2 consistent AMD risk factors (increasing age and current smoking) in our Japanese population, consistent with findings from studies elsewhere in whites.

There has long been a belief that AMD is less common in Asians than in whites.⁷ Our study in Japanese people, after age standardization and direct comparability of results with the BMES, does not support this concept, at least for early AMD. As we had a small number of late AMD cases, comparative estimates of late AMD prevalence between Japanese and whites will need confirmation in larger future studies. Our data suggest that among Japanese men, late AMD appears to be just as frequent as in all whites.

Cigarette smoking has consistently been identified as a risk factor for AMD in whites,¹¹ but this association has not previously been reported in a Japanese population-based sample.^{7,13} Although cigarette smoking has been shown to be a risk factor of AMD in a case-control study,²³ our report is the first to confirm a link between current smoking and AMD in a Japanese population-based sample.^{7,13} Current smoking remains highly prevalent among Japanese men (36.8% of participants in this study), which translates to a 66% population-attributable risk for late AMD cases in Japanese men that are attributable to their smoking behavior. As smoking is a well-recognized, modifiable AMD risk factor, smoking cessation is an important public health measure to reduce the burden of AMD, particularly among Japanese men.

The Hisayama study showed that hypertension and male gender were associated with AMD,¹³ but we could not definitively confirm these associations. Although not statistically significant, there was a suggestion of higher late AMD prevalence in men than in women. Gender differences in AMD prevalence have been inconsistently reported,²⁴ with a higher prevalence of late AMD in men reported in 2 Asian populations (the Hisayama Study¹³ and a United States Chinese population⁹). In contrast, most studies conducted in Western white populations have shown a higher prevalence of AMD in women.^{24,25} There might be several reasons for a higher prevalence of late AMD in Japanese men relative to women. First, the proportion of current smokers is much higher in Japanese men (36.8% vs. 2.8%), so this seems likely to be the main reason for the gender difference in late AMD prevalence. Because the proportion of current smoking among women was 14.4% in the BMES,²⁶ compared with only 2.8% among women of our sample, this could explain the lower prevalence of late AMD in Japanese women compared with the corresponding BMES rates. Second, it has been hypothesized that polypoidal choroidal vasculopathy (PCV), a possible variant of neovascular AMD, is relatively prevalent among Japanese, particularly men.²⁷ Maruko et al,²⁸ in a Japanese case series, reported that 54.7% of the clinical neovascular AMD cases had PCV, with over 70% of these PCV cases occurring in men. It is possible that the higher prevalence of neovascular AMD in men in our study is related to the higher frequency of PCV in Japanese men. Unfortunately, we are unable to verify this using appropriate tests (e.g., indocyanine green angiography) in our epidemiological study. Finally, differences in the genetic composition of late AMD, such as the complement factor H gene, between different racial/ethnic groups may relate to this observation.^{29,30}

Limitations and potential biases of this study should be noted. First, the overall response rate was only 53%. Of those with fundus photographs, 7.6% had insufficient quality for AMD lesion grading, mainly due to media opacity (cornea or lens) among older participants. These limitations could have introduced selection bias, resulting in either underestimation or overestimation of AMD prevalence. Second, only one nonmydriatic fundus photograph was taken from a single eye of each participant examined in the study. This is likely to have underestimated AMD prevalence by 21.5% to 45%.^{6,7,31} Nevertheless, we were able to compare the prevalence of early and late AMD in right eyes with corresponding prevalence in the BMES population, after age standardization. Third, comparisons between studies are limited because of differences in the fundus cameras used. The nonmydriatic fundus photography used in our study could have underestimated the prevalence of AMD lesions.³²

In conclusion, our Funagata study indicates an early AMD prevalence in adult Japanese similar to that of the Australian white population, after age standardization to enhance comparability. The overall prevalence of late AMD was lower but could have reflected the relatively small number of Japanese in this study over 80 years. We confirm older age and cigarette smoking as major risk factors for AMD in Japanese people. Thus, our study does not support the concept that AMD is uncommon in Japan or is unrelated to cigarette smoking.

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Correlation between visual function and photoreceptor inner/outer segment junction in patients with retinitis pigmentosa

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Abstract

Purpose To determine whether a significant correlation exists between the visual acuity or foveal thickness and the status of the inner and outer segment junction (IS/OS) of the photoreceptor in patients with retinitis pigmentosa (RP).

Methods Three hundred eyes of 163 patients with RP were examined with the optical coherence tomography (OCT). The IS/OS appeared as a distinct, highly reflective line just vitread of the retinal pigment epithelium in the OCT3 images. The IS/OS line was graded into three groups. The correlations between the grade of the IS/OS and age, best-corrected visual acuity (BCVA), and central foveal thickness (CFT) were determined.

Results Grade 1 included 93 eyes (31.0%) in which an IS/OS line was not seen, Grade 2 included 67 eyes (22.3%) with an abnormal IS/OS, and Grade 3 included 140 eyes (46.7%) with a normal IS/OS. The correlation between the IS/OS grade and age was not significant ($P = 0.5536$). The IS/OS grade was significantly correlated with BCVA and CFT (both $P < 0.0001$). The BCVA was significantly better in Grade 3 eyes than Grades 1 and 2 (both $P < 0.0001$). The CFT was significantly thinner in Grade 1 eyes than in Grades 2 and 3 (both $P < 0.0001$). In Grade 3, the mean length of the IS/OS was 2.51 ± 1.42 mm (\pm SD). The length of the IS/OS was significantly correlated with the BCVA ($P < 0.0001$, $r = -0.375$).

Conclusions The presence of the IS/OS was associated with better visual acuity and thicker fovea in RP patients. The absence of an IS/OS may reflect a foveal dysfunction in RP patients.

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Keywords: photoreceptor inner and outer segment junction; retinitis pigmentosa; visual function; optical coherence tomography

Introduction

Retinitis pigmentosa (RP) is a slowly progressive retinal disease and patients with RP show reduced visual function, mainly by degeneration of the rod and cone photoreceptors and the retinal pigment epithelium (RPE).^{1–3} Several different genetic mutations have been found to be associated with RP, although RP patients can have similar symptoms and retinal histopathology.^{1–3} The degeneration of the retina usually starts in the mid-periphery, leading to nyctalopia. When the disease is advanced, the retinal degeneration gives rise to a characteristic ring-shaped scotoma that expands to the periphery and macula.

The earliest histopathological changes in all forms of RP is a shortening of the photoreceptor outer segments.³ The loss of the cones reduces central vision at the end stage of disease.^{2,3} Therefore, morphological assessments of the photoreceptors in the macular area can be useful in estimating the residual central visual function in RP patients.

Optical coherence tomography (OCT) is a well-established method of examining the retinal architecture *in vivo*. Recently, a distinct, highly reflective line was detected just vitread of the RPE layer in the images obtained by the

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ultrahigh-resolution OCT (UHR-OCT).^{4,5} This line has been identified as the inner/outer segment junction (IS/OS) of the photoreceptor, and a distinct and continuous line indicated normal alignment of membranous discs in the photoreceptor outer segments.⁶ Because the alignment of the discs is necessary for the normal functioning of the photoreceptors, the presence of a normal IS/OS line in the OCT images most likely indicates normally functioning photoreceptors. It has also been reported that the presence of the IS/OS line in the OCT images is correlated with the recovery of good vision after macular hole surgery and is essential for normal visual function.⁷⁻⁹ At present, it is generally accepted that the OCT3 images correspond with the UHR-OCT images in major parts,^{6,10} and the IS/OS is so distinct that it can be observed in the OCT3 images as well as in the UHR-OCT images.⁶⁻¹¹

In RP patients, several OCT studies have been conducted on the correlation between the retinal structure and visual function.¹¹⁻¹³ Sandberg *et al*¹² examined the third high-reflectance band that represented the IS/OS in RP patients using OCT3 (Zeiss Humphrey, Sun Leandro, CA). They examined the IS/OS only in the central 1 mm area and examined the IS/OS using false-colour images in which the IS/OS line is sometimes difficult to differentiate from the RPE layer, especially in the high-signal areas of the OCT images.¹⁴ Witkin *et al*¹¹ reported the central foveal thickness (CFT) and foveal outer segments thickness were associated with the visual acuity. Their OCT images were obtained with UHR-OCT, but only nine RP patients were studied.

The purpose of this study was to determine whether a significant correlation is present between the visual acuity or foveal thickness and the grade of the IS/OS line observed in the OCT3 greyscale images in a large number of RP patients. We also performed a quantitative analysis of the relationship between the length of the IS/OS and the visual acuity.

Subjects and methods

This observational, cross-sectional case study was performed on 300 eyes of 163 patients with a diagnosis of RP (81 women and 82 men). The study was conducted at Chiba University Hospital between December 2003 and April 2007. RP patients were diagnosed based on the clinical history, funduscopy appearance, visual field testing, fluorescein angiography, and full-field electroretinograms recorded under ISCEV-standardized conditions. Atypical RP cases such as sector RP and unilateral RP were excluded. Eyes with an epiretinal membrane, macular oedema, poor fixation because of extremely low visual acuity (<0.01), myopic eyes with posterior staphyloma, and eyes with opacities in the

media that affected the visual acuity were excluded. All subjects underwent a standard ophthalmological examination, including best-corrected visual acuity (BCVA) measurements, applanation tonometry, slit-lamp biomicroscopy, indirect ophthalmoscopy, and colour fundus photography. The BCVA was measured with a Japanese standard Landolt visual acuity chart and converted into logarithm of the minimal angle resolution (logMAR) units for statistical analyses.

Retinal tomographic images were obtained with an OCT3 with 5-mm horizontal scans through the fovea. All OCT images were acquired through a dilated pupil. The greyscale images were used for a more precise identification and measurement of the IS/OS.¹⁴ The appearance of the IS/OS in the OCT images at the fovea was graded from 1 to 3: Grade 1, IS/OS not visible; Grade 2, abnormal IS/OS (Figure 1); and Grade 3, normal IS/OS (Figure 2).¹² To grade the cases, we measured the length of the IS/OS, which extended from just beneath the fovea, and defined eyes as Grade 3 when the length of the continuous IS/OS line was >0.5 mm. The eyes with disruptions of the IS/OS just beneath the fovea were placed in Grade 2. The grade of the IS/OS was confirmed by two of the authors (SA and TB) who were masked to the visual acuity.

The CFT was measured manually on the horizontal OCT3 images of all eyes at the thinnest point of the fovea. The distance from the innermost layer of retina to the outer border of the RPE was measured.

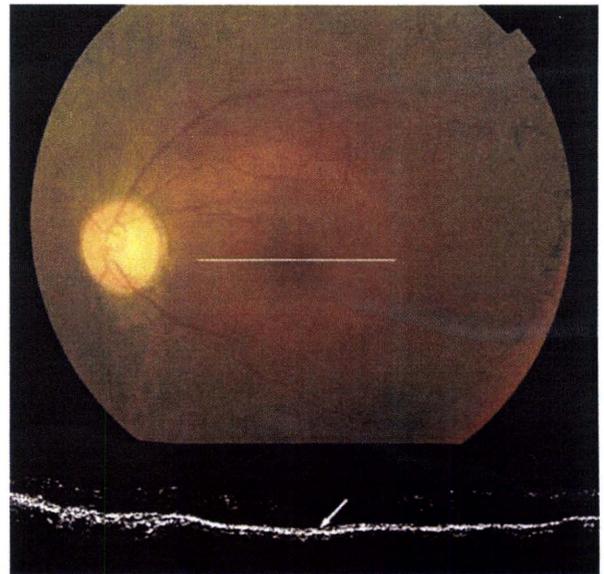


Figure 1 An eye classified as Grade 2 with an abnormal photoreceptor IS/OS. Top: fundus photograph of a 45-year-old man. The white line indicates the direction of the OCT scan. BCVA was 0.4. Bottom: greyscale OCT image of a 5-mm horizontal scan. A disrupted IS/OS line (white arrow) can be seen.

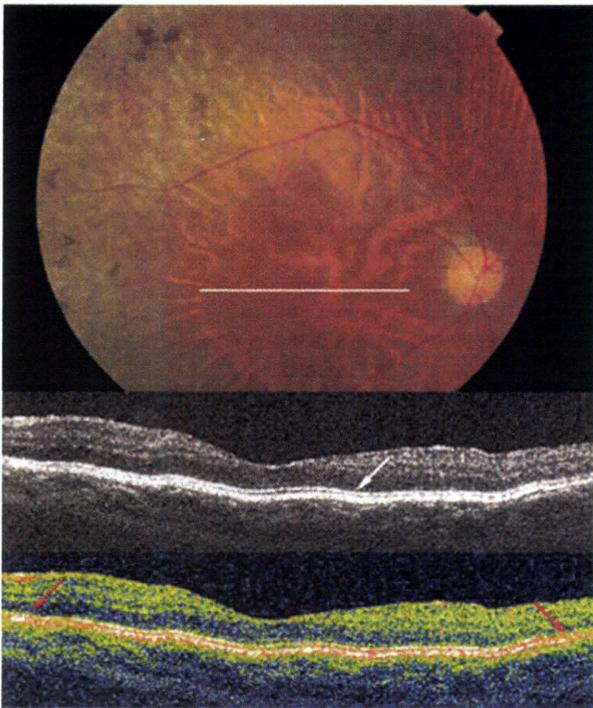


Figure 2 An eye classified as Grade 3 with a normal photoreceptor IS/OS. Top: fundus photograph of 35-year-old man. The white line indicates the direction of the OCT scan. BCVA was 1.0. Middle: greyscale OCT image of a 5-mm horizontal scan. Foveal thickness (central foveal thickness: 207 μm) was within normal limits, and the presence of a normal IS/OS line (white arrow), which covered the entire scan length, can be seen. Bottom: false-colour OCT image. The IS/OS line is difficult to differentiate from the RPE layer in some high-signal areas (red arrows).

The significance of the differences of age, BCVA, and CFT in the three IS/OS grades were tested statistically using one-factor ANOVA. When the three groups were compared independently, Fisher's protected least significant difference (PLSD) was used. The correlations between the length of the IS/OS and BCVA, age, or CFT were examined by Pearson's correlation tests. A P -value < 0.05 was considered statistically significant.

Results

The IS/OS was not detected (Grade 1) in 93 eyes (31.0%; Table 1), an abnormal IS/OS (Grade 2) was detected in 67 eyes (22.3%), and the IS/OS was normal (Grade 3) in 140 eyes (46.7%).

The mean age of all the RP patients was 50.5 ± 13.1 (\pm SD) years with a range of 11–75. The mean age was 51.3 ± 12.1 years in Grade 1, 50.6 ± 14.5 years in Grade 2, and 49.4 ± 13.0 years in Grade 3 group (Table 1). The differences in the three groups were not significant ($P = 0.5536$, one-factor ANOVA).

Table 1 Number of eyes, age, BCVA, and CFT in IS/OS subgroup

Grade ^a	Number of eyes (%)	Age (years) ^b	BCVA (logMAR) ^b	CFT (μm) ^b
1	93 (31.0%)	51.3 ± 12.1	0.83 ± 0.55	152 ± 40
2	67 (22.3%)	50.6 ± 14.5	0.31 ± 0.29	199 ± 42
3	140 (46.7%)	49.4 ± 13.0	0.03 ± 0.12	202 ± 34
		$P = 0.5536$	$P < 0.0001$	$P < 0.0001$

IS/OS = photoreceptor inner and outer segment junction; BCVA = best-corrected visual acuity; CFT = central foveal thickness.

^aThe IS/OS was classified as Grade 1 (absence), Grade 2 (abnormal), and Grade 3 (normal).

^bThe data are displayed as means \pm SD.

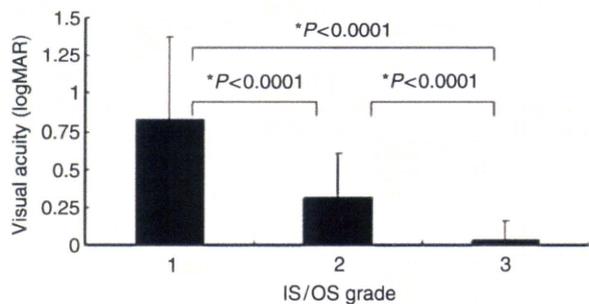


Figure 3 Mean visual acuity (logMAR units) as a function of the grade of the photoreceptor IS/OS. The difference among the three groups is statistically significant ($P < 0.0001$). * P : difference between the two groups.

The mean BCVA was 0.83 ± 0.55 logMAR units in the Grade 1 group, 0.31 ± 0.29 U in Grade 2 group, and 0.03 ± 0.12 U in Grade 3 group (Table 1). The differences among the three groups were significant ($P < 0.0001$, one-factor ANOVA). When the mean BCVA of the three groups was compared independently with each of the other groups, the BCVA was significantly better in Grade 3 than in Grades 1 and 2 (both $P < 0.0001$, Fisher's PLSD; Figure 3). The BCVA was also significantly better in Grade 2 than in Grade 1 ($P < 0.0001$, Fisher's PLSD).

The mean CFT was 152 ± 40 μm in Grade 1, 199 ± 42 μm in Grade 2, and 202 ± 34 μm in Grade 3 (Table 1). There was a significant difference among the three groups ($P < 0.0001$, one-factor ANOVA). The mean CFT was significantly thinner in Grade 1 compared with the other two groups (both $P < 0.0001$, Fisher's PLSD). However, the difference between Grades 2 and 3 was not significant ($P = 0.6417$, Fisher's PLSD).

The length of the IS/OS line was measured in eyes in the Grade 3 group. The mean IS/OS length was 2.51 ± 1.42 mm, and the IS/OS line was detected over the entire scan length of 5 mm in 17 (12.1%) of the 140 eyes.

The correlation between the length of the IS/OS and the BCVA was significant ($r = -0.375$; $P < 0.0001$,

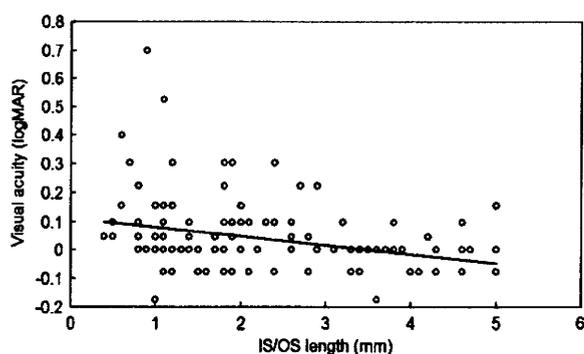


Figure 4 Correlation between the length of the photoreceptor IS/OS and visual acuity (logMAR units). The IS/OS length is significantly correlated with the visual acuity ($r = -0.375$, $P < 0.0001$). The solid line represents the linear regression line; $y = 0.112 - 0.032x$.

Pearson's correlation tests; Figure 4). However, the correlations between the IS/OS length and age or CFT were not significant ($r = 0.037$, $r = 0.141$, respectively; $P = 0.6632$, $P = 0.0968$, respectively, Pearson's correlation tests).

Discussion

RP primarily affects the photoreceptor and the RPE. The earliest histopathological change in the photoreceptors is a shortening of the rod outer segments. This is progressive and eventually results in the death of the rods. The death of the rods is usually accompanied by changes in the cones, especially a shortening of their outer segments.² Therefore, an assessment of the outer segment is essential in estimating the damage of the photoreceptors.

The IS/OS line was clearly delineated in the OCT3 greyscale images in this study. An OCT image is usually examined with false-colour images, but the IS/OS line is sometimes difficult to differentiate from the RPE layer, especially in high-signal OCT images (Figure 2). In such cases, greyscale images are better because they can enhance each layer, and a distinct image of the IS/OS line separated from the RPE layer can be seen.¹⁴ Ishikawa *et al*¹⁴ examined eyes with cystoid macular oedema using both false-colour and greyscale OCT images and concluded that greyscale images showed a finer gradation of signal reflectance. This finer gradation of greyscale images is mainly because the false-colour algorithm converts tissue reflectivity into intensity in an abrupt, linear, and stepwise fashion, whereas the greyscale algorithm uses a smooth curved line (square root) for conversion.¹⁴ Thus, we recommend that the greyscale image should be used as a standard method to identify and study the IS/OS line.

We were not able to detect an IS/OS line in 31.0% of the eyes, able to detect an abnormal IS/OS in 22.3% of the eyes, and a normal IS/OS in 46.7% in the OCT3 greyscale image in the 163 RP patients. On the other hand, Sandberg *et al*¹² detected no IS/OS (third high-reflectance band) line in 29.5%, an abnormal IS/OS in 56.6%, and a normal IS/OS in 13.9% in OCT3 false-colour images of 162 RP patients. Because of the differences in age, gender, duration of disease, and type of RP between the two studies, the results of these two studies cannot be simply compared. However, there is a possibility that our higher detection rate was due to the use of greyscale images.

Our results showed that the IS/OS grade was highly correlated with the BCVA in our RP patients. These findings are in agreement with the report by Sandberg *et al*¹² that shows that the IS/OS grade was significantly correlated with the visual acuity and with the CFT. On the other hand, Witkin *et al*¹¹ reported that the foveal outer segment/pigment epithelium thickness was highly correlated with the visual acuity in RP patients. Considering these results, we suggest that the presence of the IS/OS indicates a preservation of foveal function.

Ergun *et al*¹⁵ quantified the transverse photoreceptor loss at the level of the photoreceptor outer segments, that is, a disruption in the length of the IS/OS line, in patients with Stargardt's disease using UHR-OCT. They reported that the transverse photoreceptor loss was significantly correlated with the visual acuity, central atrophy determined by fluorescein angiography, and the degree of autofluorescence. In our study, we examined IS/OS length or transverse photoreceptor presence in RP patients. Our finding that the IS/OS length was correlated with the BCVA is in agreement with their findings. Together, these results suggest that examining the IS/OS length is a useful way to assess the visual function of photoreceptor diseases such as Stargardt's disease and RP.

The presence of the IS/OS line was not correlated with patient's age. This does not mean that the degenerative changes were not related to the duration of the disease. We suggest that our results were obtained from different types of RP patients, at different stages, and with different rates of degeneration, and these differences accounted for the nonsignificant correlation between the presence of the IS/OS and age.

We found a significant difference in the CFT between Grades 1 and 2 and between Grades 1 and 3, but not between Grades 2 and 3. It has been reported that a foveal thinning measured with the OCT was correlated with the visual acuity in patients with RP and Stargardt's disease.^{11,12,15} Witkin *et al*¹¹ reported that the foveal thinning might be primarily due to photoreceptor loss. In our RP patients, Grade 2 eyes had a fovea as thick as that of eyes in Grade 3, and we assumed that the damage of

the photoreceptor in Grade 2 eyes was partial, and that the length of the outer segments was preserved. It should be noted that OCT3 has an axial imaging resolution of approximately 10 μm , whereas the UHR-OCT has an axial imaging resolution of approximately 3 μm . This difference may be another reason why we were not able to find a difference in the CFT between eyes in Grades 2 and 3 using the OCT3 images.

One of the limitations of this study was its cross-sectional design. We were not able to determine the time course of the changes in the IS/OS. To determine this, a follow-up longitudinal study will be required.

We conclude that the presence of the IS/OS is associated with better visual acuity and normal foveal thickness in RP patients. Our findings show that OCT can assess the photoreceptor cells *in vivo*, and the significant correlation between the visual acuity and presence or length of the IS/OS indicates that the IS/OS line may be an important parameter to monitor RP patients.

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Clinicopathologic Findings in Polypoidal Choroidal Vasculopathy

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PURPOSE. To elucidate the pathogenic mechanism of polypoidal choroidal vasculopathy (PCV) based on histopathologic findings.

METHODS. Specimens obtained by surgical excision of PCV from five eyes of five patients (mean age, 75.6 ± 3.1 years) were studied histopathologically. Immunohistochemical studies were also performed to identify CD34, vascular endothelial growth factor (VEGF), CD68, α -smooth muscle actin (α -SMA) and hypoxia-inducible factor (HIF)-1 α .

RESULTS. Hyalinization of choroidal vessels and massive exudation of fibrin and blood plasma were observed in all the specimens of PCV lesions. Some blood vessels were located above the RPE in two of the five eyes. Immunohistochemically, CD68-positive cells were detected around the hyalinized vessels. There were no α -SMA-positive cells in the vessels of PCV. CD34 staining showed endothelial discontinuity. Vascular endothelial cells within the PCV specimens were negative for VEGF. HIF-1 α positive inflammatory cells were located in the stroma of specimens.

CONCLUSIONS. Hyalinization of choroidal vessels, like arteriosclerosis, is characteristic of PCV. (*Invest Ophthalmol Vis Sci*. 2008;49:4729–4737) DOI:10.1167/iovs.08-2134

Polypoidal choroidal vasculopathy (PCV) is a disorder characterized by vessel networks and apical polypoidal lesions.¹ PCV is known to be more common in non-white populations (including blacks, Hispanics and Asians).² The incidence of PCV in Japanese has also been reported to be remarkably high.^{3,4} There are two opinions on the pathogenesis of PCV: inner choroidal vessel abnormalities^{1,5–12} and variants in choroidal neovascularization (CNV).^{13–17} Although several pathologic studies have focused on PCV, the pathogenesis is still unclear.^{10–16,18}

Recently, as PCV has become better recognized, it has become apparent that some specimens excised under a diag-

nosis of age-related macular degeneration (AMD) included PCV. We examined the histopathology of five PCV specimens from five eyes, the specimens having been excised between 2001 and 2003, with a diagnosis of neovascular AMD. Judged against the PCV diagnostic criteria which were subsequently introduced in 2005,¹⁹ these five specimens were obtained from definite cases of PCV. Our findings shed light on the pathogenesis of PCV.

MATERIALS AND METHODS

We studied specimens surgically extracted from five eyes of five patients (four men and one woman, 71–79 years of age, mean 75.6 ± 3.1) with PCV. A diagnosis of neovascular AMD was made based on fluorescein angiography (FA) and clinical findings between 2001 and 2003. Although indocyanine green angiography (IGA) showed polypoidal lesions in all five eyes, no typical network vessels were observed. However, PCV was diagnosed based on recently published criteria used to identify PCV¹⁹ and interpret FA findings.²⁰

Informed consent for the surgical procedure and for the use of excised tissue was obtained from all patients, in accordance with the tenets of the Declaration of Helsinki. Surgical excision of subfoveal CNV was performed according to the method of Lambert et al.²¹ The surgical specimens were immediately fixed in 10% formalin in phosphate-buffered solution (pH 7.4) and embedded in paraffin, and 4- μ m serial sections were prepared and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) for basement membranes, phosphotungstic acid hematoxylin (PTAH) for fibrin, and elastic van Gieson for elastic fibers.

For immunohistochemical studies, paraffin-embedded sections were deparaffinized, hydrated and rinsed in deionized water. Immunostaining was performed using an automated immunostaining machine (Ventana Medical Systems, Inc. Tucson, AZ) with Endogenous Biotin Blocking Kits (Ventana Medical Systems). The primary antibodies used were as follows. Anti-CD34 antibody (monoclonal mouse anti-human CD34, clone QBEnd-10; 1:20; Dako Cytomation, Carpinteria, CA) was used to confirm blood vessels in the specimens. Anti- α -smooth muscle antibody (Anti-actin, smooth muscle monoclonal clone; 1:15; Thermo Fisher Scientific Inc., Waltham, MA) was used to identify smooth muscle cells and myofibroblasts. Anti-vascular endothelial growth factor (VEGF) antibody (rabbit polyclonal antibody; 1:50; Santa Cruz Biotechnology, Santa Cruz, CA) was used to demonstrate localization of VEGF as an angiogenic factor. Anti-CD68 antibody (mouse monoclonal anti-human macrophage, clone PG-M1; 1:80; Dako Cytomation) was used to identify macrophages. Anti-hypoxia inducible factor (HIF)-1 α antibodies (rabbit antibodies; 1:50; Chemicon International, Temecula, CA) were used to examine the oxidative states of tissues. Negative controls were obtained by omitting the primary antibodies. Subsequent reactions with secondary antibodies and visualization were achieved with DAB (DAB Universal Kit; Ventana Medical Systems). The sections were counterstained with hematoxylin and mounted (Permount; Fisher Scientific, Pittsburgh, PA). All the stained slides were evaluated histologically by light microscopy (VANOX-S; Olympus, Tokyo, Japan).

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TABLE 1. Patient Characteristics and Immunohistopathologic Characteristics of PCV Lesion

Case	Age (y)	Sex	History	Size (DD)	Exudative Change	Relations of PCV and RPE	α SMA (Vascular Channel)	VEGF (Endothelium)	CD68	HIF-1 α
1	77	F	Hypertension/rheumatoid arthritis	2.2	Prominence	Beneath RPE	-	-	+	+
2	71	M	None	1.1	Prominence	Above RPE (combined type 1 CNV)	-	-	+	+
3	74	M	Hypertension/hyperlipidemia	1.7	Prominence	Beneath RPE	-	-	+	+
4	77	M	Hyperlipidemia	1.4	Prominence	Above RPE (combined type 1 CNV)	-	-	+	+
5	79	M	None	2.0	Prominence	Beneath RPE	-	-	+	+

-, Not present; +, present.

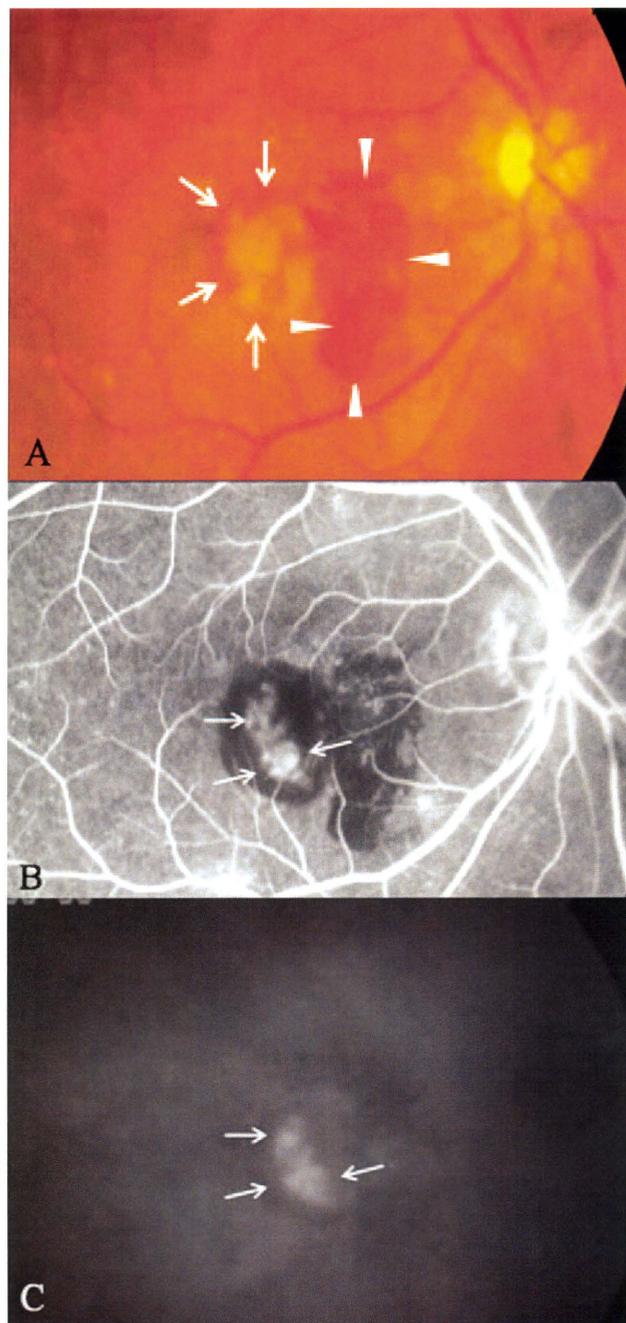


FIGURE 1. (A) Color fundus photograph showing a white fibrin-like lesion (arrow) adjacent to subretinal hemorrhage (arrowhead) and serous retinal detachment in the macula. (B) Fluorescein fundus angiography showing granular hyperfluorescence in the early phase (arrow). (C) IGA showing polypoidal lesions resembling grape clusters.

RESULTS

The clinical characteristics of the patients are summarized in Table 1. Three of the five patients had systemic diseases including hypertension and hyperlipidemia. In this study, PCV lesion sizes were relatively small in all cases; from 1.1 disc diameters (DD) to 2.2 DD, as measured on IGA photographs. The pathologic findings revealed little granulation tissue formation in any of the specimens. On the other hand, all the specimens exhibited massive exudative change and leak-

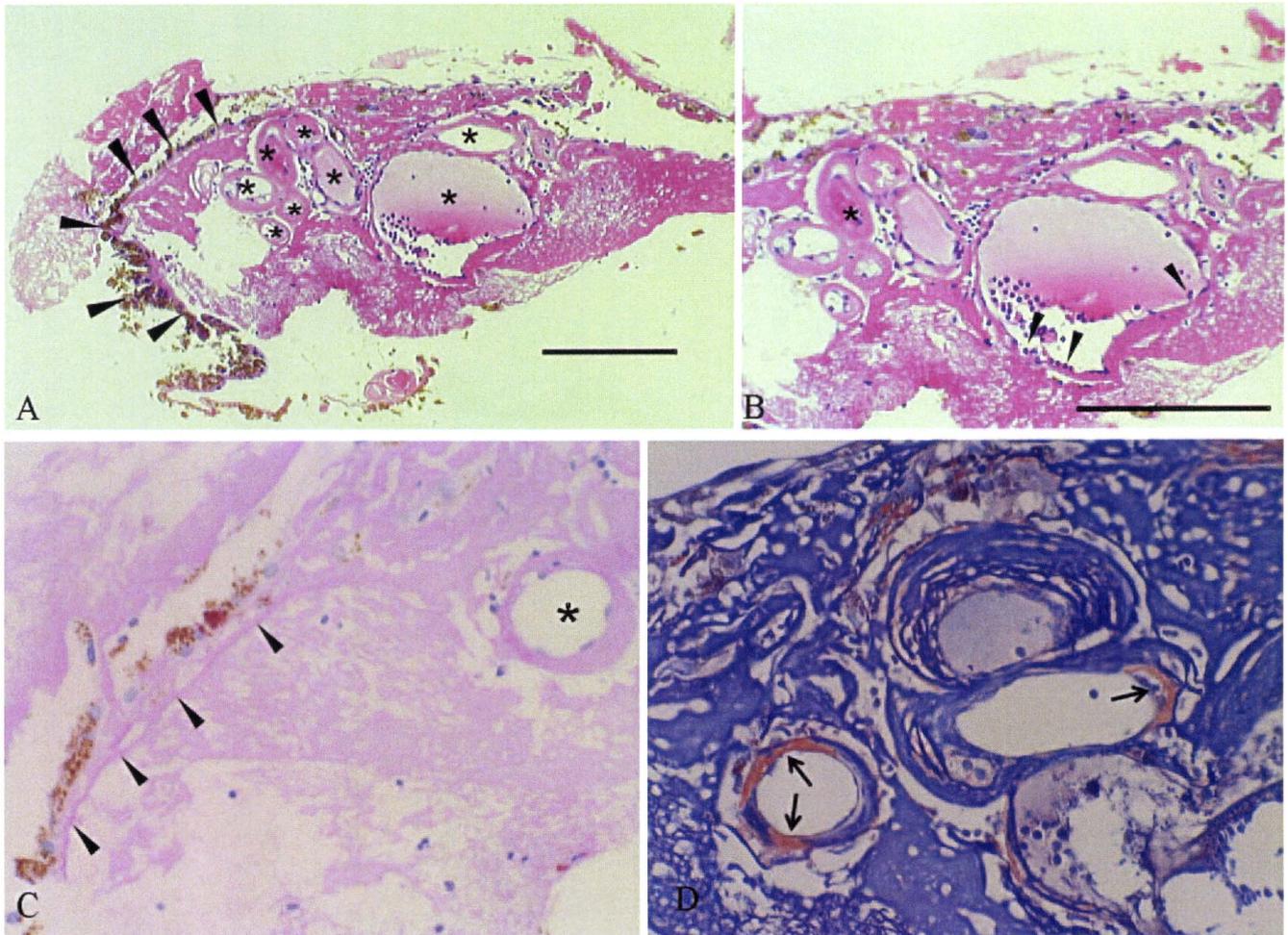


FIGURE 2. (A) Photomicrograph showing abnormally dilated vessels (*) beneath the RPE (arrowhead). The walls of these vessels were thickened and hyalinized, owing to extravasation of plasma protein and deposition of basement membrane-like material (hematoxylin-eosin). (B) Higher magnification of hyalinized vessels indicated in (A). There were numerous blood cells in the vascular cavity and several neutrophils adhered to the inner vessel walls (arrow). (*) Obstruction of hyalinized vessels. The RPE overlying hyalinized vessels was obscured. The diameter of the most dilated vessel exceeds 250 μm . (C) Bruch's membrane is stained by PAS (arrowhead). A hyalinized vessel (asterisk) is located beneath Bruch's membrane (periodic acid-Schiff). (D) Hyalinized vessels showing fibrosis (red) and exudative material (blue) (phosphotungstic acid hematoxylin). Scale bar, 200 μm ; magnification: (A) $\times 25$; (B) 50; (C, D) 100.

age of fibrinous material. The continuity of the retinal pigment epithelium (RPE) above or beneath the vessels had been lost in most cases, and blood vessels were confirmed to be located above the RPE in two of the five specimens. Furthermore, all the vessels exhibited hyalinization, choriocapillaris had disappeared, even in the cases in which RPE had been preserved.

Immunohistochemically, CD68-positive foamy macrophages were detected around the hyalinized vessels, but α -smooth muscle actin (SMA)-positive cells were not. CD34 staining revealed discontinuity of the endothelial lining of these abnormal vessels.

Myofibroblasts, recognized by their fibroblast-like appearance and α -SMA immunoreactivity were seen in the stroma of several specimens. Although VEGF-positive cells were detected among CD68-positive foamy macrophages, fibroblast-like cells and RPE cells, vascular endothelial cells in the PCV specimens were negative for VEGF. HIF-1 α -positive cells were located in the stroma of specimens.

Angiographic and histopathologic findings in three of our cases are presented in the following sections.

Case 1

The patient was a 77-year-old woman with hypertension and rheumatoid arthritis. Preoperative visual acuity was 0.3 in the right eye in December 2000. As the white lesion was situated above the RPE with subretinal hemorrhage (Fig. 1A), and classic CNV was identified on FA (Fig. 1B), the white lesion was thought to be subretinal CNV. Although IGA showed several small polypoidal lesions resembling clusters of grapes (Fig. 1C), a diagnosis of neovascular AMD was made based on ophthalmoscopic and FA findings. CNV excision was performed in June 2001.

The specimen contained abnormally dilated vessels beneath the RPE, and the vessel walls were thick and hyalinized, because of extravasation of plasma protein and deposition of basement membrane-like material (Fig. 2A). The RPE overlying the dilated vessels was thus obscured. There were numerous blood cells in the vascular cavity, and several neutrophils adhered to the inner vessel walls suggesting stagnation of blood. Obstruction of the hyalinized vessels was also observed. The diameter of the most dilated vessel exceeded 250 μm (Fig. 2B). PAS staining revealed hyalinized vessels beneath Bruch's

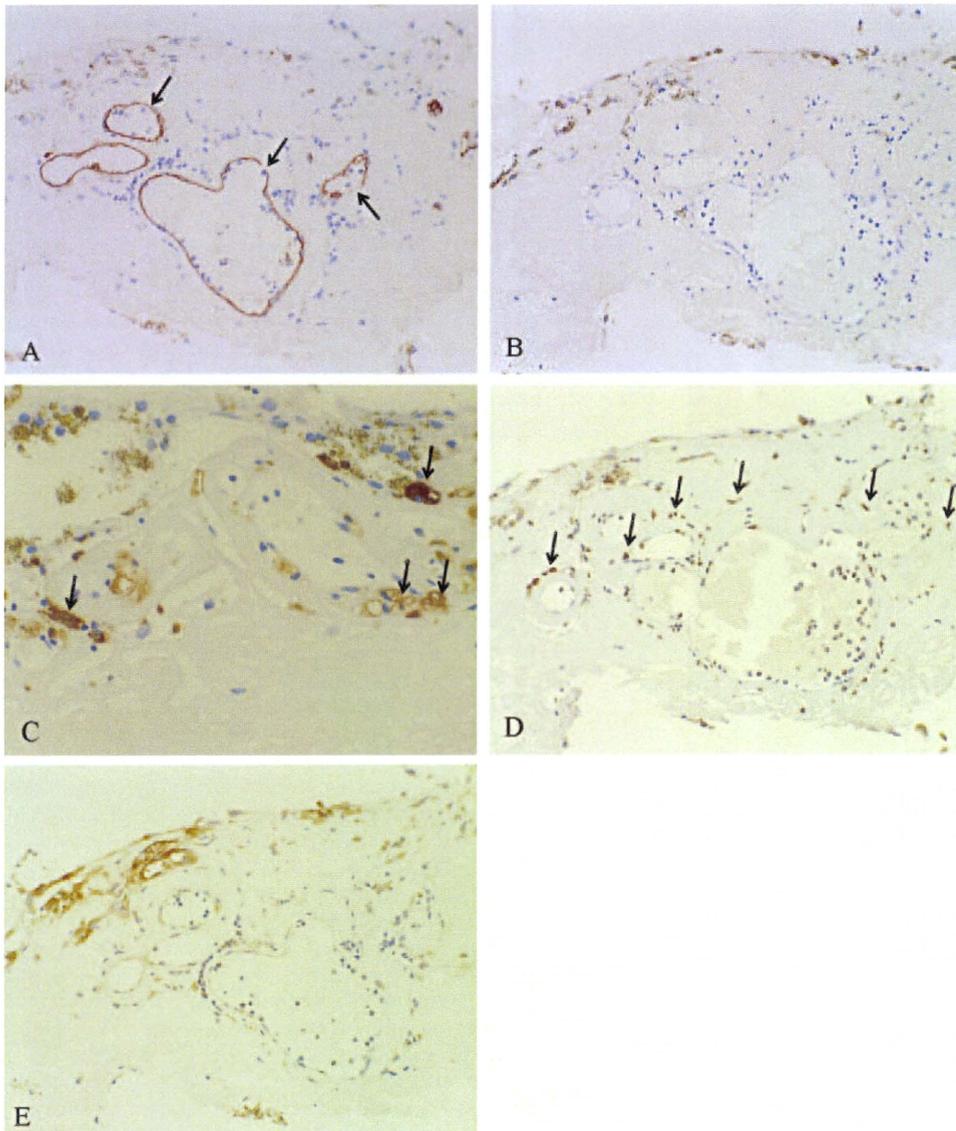


FIGURE 3. Immunohistochemistry of PCV. (A) Vascular endothelium expressing the CD34 marker. Note discontinuous endothelium stained with CD34 (arrow). (B) α -SMA staining was negative in the hyalinized vessels. (C) Macrophages expressing the CD68 marker. Note the presence of CD68-positive foamy macrophages around dilated vascular cavities and occasionally within vessel walls. (D) Immunohistochemistry for HIF-1 α . There was distinct staining of nuclei, mainly in mononuclear cells (arrow). (E) Vascular endothelium is negative for VEGF staining. Magnification: (A, B, D, E) $\times 50$; (C) $\times 100$.

membrane (Fig. 2C). PTAH staining revealed significant hyalinization of these vessels with marked extravasated plasma protein (Fig. 2D). The abnormal vessels were lined with endothelium stained by CD34, which revealed discontinuity in the vascular endothelium (Fig. 3A). There were no α -SMA-positive cells in the hyalinized vessels (Fig. 3B). Macrophages staining positively for CD68 had infiltrated around the dilated vascular cavity, and foamy macrophages were occasionally seen within the vessel walls (Fig. 3C). HIF-1 α -positive mononuclear cells were located in the stroma of specimens (Figs. 3D). Although immunoreactive deposits of VEGF were present at the macrophages, fibroblast-like cells, and RPE cells, the vascular endothelial cells were negative for VEGF (Fig. 3E).

Case 2

The patient was a 71-year-old man with no systemic disease. Preoperative visual acuity was 0.07 in the right eye in November 2002. Two orange lesions, presumably polypoidal, surrounded by a white lesion possibly indicating accumulation of fibrin material, were recognized in the macular area of the right eye (Fig. 4A). FA revealed two small hyperfluorescent areas and

a hyperfluorescent area indicating pigment epithelial detachment (PED; Fig. 4B). The IGA revealed hyperfluorescent areas apparently representing polypoidal lesions and a hypofluorescent area indicating PED (Fig. 4C). CNV excision was performed in March 2003.

The specimen contained abnormally dilated vessels above the RPE, and these vessels were hyalinized. Exudative change around the vessels was significant. This lesion was thought to be a portion of a PCV. On the other hand, fibrovascular tissues with marked fibrosis, small vascular channels, and less exudative changes were identified beneath the RPE, and were thought to represent CNV (Fig. 5A). Although occult CNV was apparently not observed by FA, it was thought to have existed beneath the PED. This specimen was considered incidentally to include both PCV and CNV lesions. PTAH staining clarified the difference between PCV and CNV (Fig. 5B). In the PCV portion, defects in the vascular endothelium were revealed by CD34 staining (Fig. 6A), whereas the continuity of the endothelium was maintained in the CNV portion (Fig. 6B). Although no α -SMA-positive cells were detected in the hyalinized vessels located on the PCV portion (Fig. 6C), α -SMA-positive pericytes were observed in vascular channels located on the CNV por-

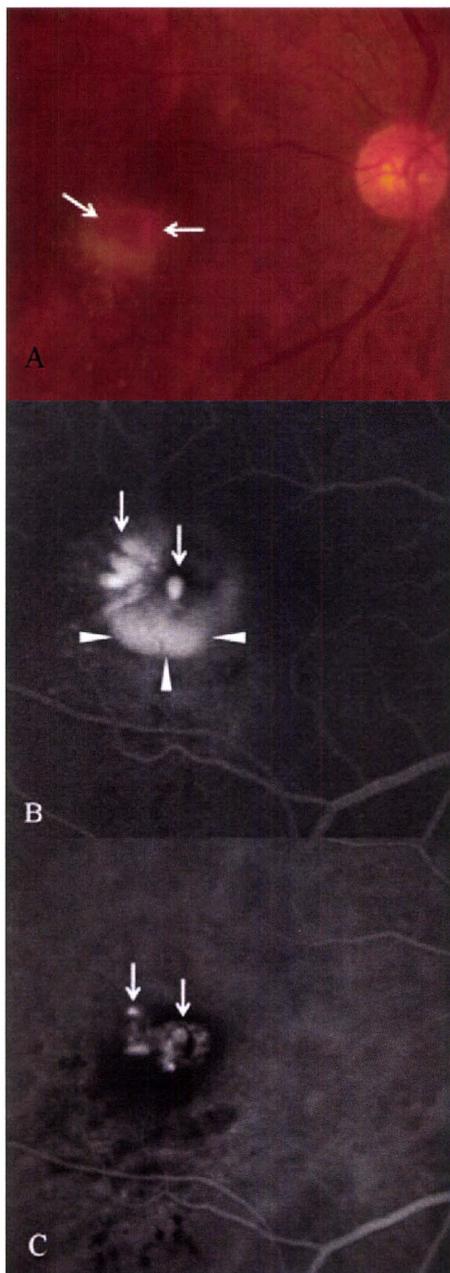


FIGURE 4. (A) Color fundus photograph shows orange lesions (*arrow*) surrounded by a white lesion. These findings are consistent with PCV (i.e., a polypoidal lesion accompanied by fibrin). (B) Fluorescein fundus angiography showing two small round hyperfluorescent lesions near the fovea (*arrow*) and a hyperfluorescent lesion indicating pigment epithelial detachment (*arrowhead*). (C) IGA showing polypoidal lesions corresponding to hyperfluorescent lesions on fluorescein fundus angiography.

tion (Fig. 6D). Foamy macrophages stained positively for CD68 had infiltrated around the dilated vascular cavity in the PCV portion (Figure 7A) and in the stroma of the CNV portion (Fig. 7B). HIF-1 α -positive cells were located in the stroma of both portions of the specimen (Figs. 7C, 7D). Although immunoreactive deposits of VEGF were present at macrophages, fibroblast-like cells, and RPE cells, vascular endothelial cells in both the PCV and the CNV portions were negative for VEGF (Figs. 7E, 7F).

Case 3

The patient was a 77-year-old man with hyperlipidemia. Preoperative visual acuity was 0.06 in the left eye in December 2000. IGA revealed a polyplike dilatation at the macula (Fig. 8A). Although the dilatation of vessels was not apparent in the specimen, many hyalinized vessels with surrounding exudative change were prominent above the RPE. The large number of vessels resembled a coil-like configuration. This lesion was thought to have a PCV portion. In contrast, fibrovascular tissues and small vascular channels were recognized beneath the RPE and were thought to represent occult CNV. Exudation and hyaline change of the vascular wall were not significant in this CNV portion. This specimen was considered incidentally to include PCV and CNV portions, as in Case 2. Furthermore, an RPE break was also identified in this specimen (Fig. 8B). PTAH staining clarified the difference between PCV and CNV (Fig. 8C). Immunohistopathologic findings were similar to those in case 1.

DISCUSSION

In this study, extensive exudative change and hyalinization of vessels were documented.

There are several reports suggesting the presence of hyalinized vessels in PCV specimens. Okubo et al.¹⁰ suggested histopathologic similarities between branched vein retinal occlusion and PCV, and the walls of the vessels in their cases had a hyaline-like appearance. Kuroiwa et al.¹¹ also reported histopathologic features of surgically excised specimens from five patients with PCV. In their series, large choroidal arterioles with an inner elastic layer were described, and the walls of these arterioles were thick and showed sclerotic change associated with an increase in basement membrane-like materials together with collagen fibers. Terasaki et al.¹³ suggested the vascular components of PCV to represent subretinal neovascularization; however, they also noted massive leakage of fibrin material around the vessels. These reports were thought to suggest the insudative and transudative hyalinization of vessels in PCV specimens.

The term hyalinization refers to extensive replacement of the smooth muscle component by amorphous pseudocollagenous tissue of a poorly defined nature.²²⁻²⁵ Hyalinized vessels are characterized by extravasation of plasma protein and deposition of basement membrane-like material. In other words, hyalinization is one of the arteriosclerotic changes seen not only in choroids, but also in other parts of the body, for instance, the brain, kidneys, and pancreas.^{26,27}

PCV is reportedly similar to retinal arterial macroaneurysm (RAM) as regards the epidemiologic associations in the following categories of patients: female, hypertensive, black, and elderly.²⁸ Two cases of PCV with RAM have been reported by Ross et al.²⁸

The histopathologic findings of retinal macroaneurysms have been extensively described by Fichte et al.²⁹ Aneurysmal sites typically show thickening of the vessel wall secondary to a fibrin-laminated clot formation with accompanying hypertrophy of the muscularis.²⁹ Hyaline, hemorrhages, and, occasionally, foamy macrophages may be seen in the vessel wall.²⁹ These findings are structurally similar to the histopathologic characteristics of PCV in our study. This may mean that PCV is closely associated with arteriosclerotic changes.

Pathologic comparison of CNV and PCV is important for understanding the pathogenesis of PCV. Case 2 included both PCV and CNV portions in the same specimen, and thus this case was thought to be appropriate for comparing the structural differences between PCV and CNV. The CNV portion

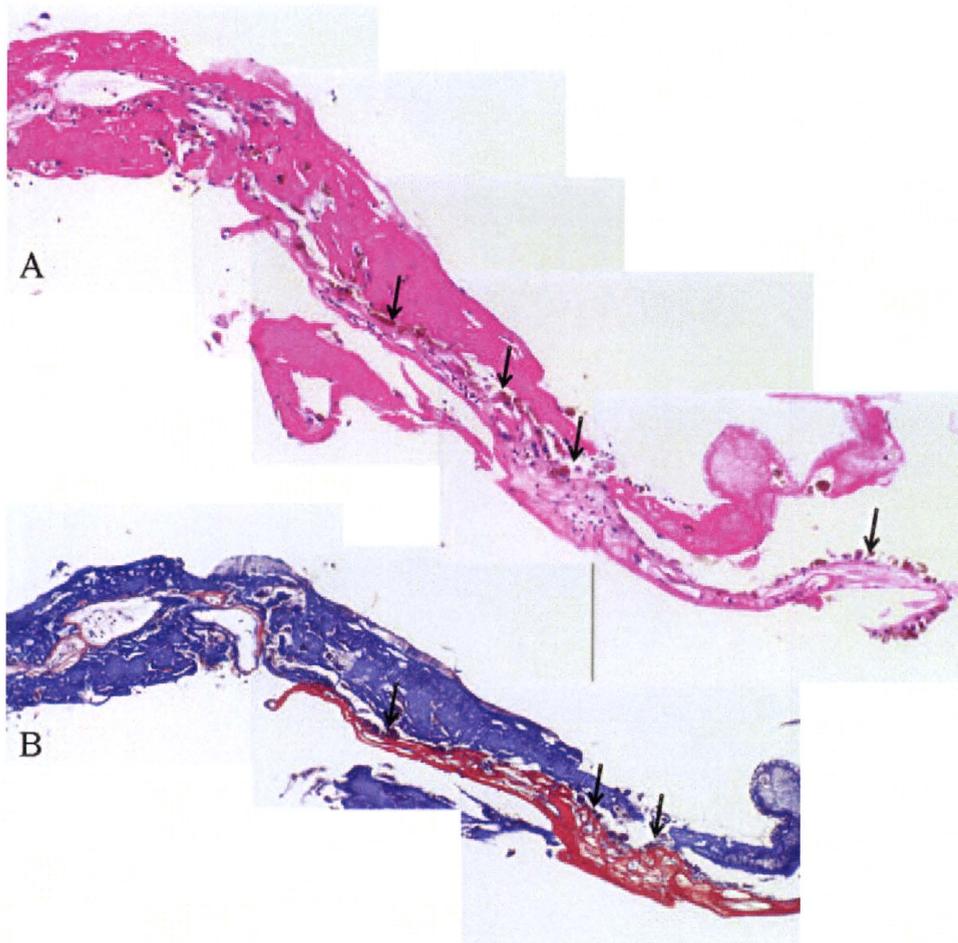


FIGURE 5. (A) *Top*: above the RPE (*arrow*), including abnormally dilated hyalinized vessels, thought to be a PCV specimen. *Bottom*: beneath the RPE which contained fibrovascular tissues with fibrosis, fibroblast-like cells, and small vascular channels, was thought to be a CNV specimen (hematoxylin-eosin). (B) *Top*: PCV staining (*blue*) indicated marked exudative change; *bottom*: CNV staining with PTAH (*red*) indicated no marked exudative change in fibrous tissue. *Arrows*: RPE. Original magnification, $\times 50$.

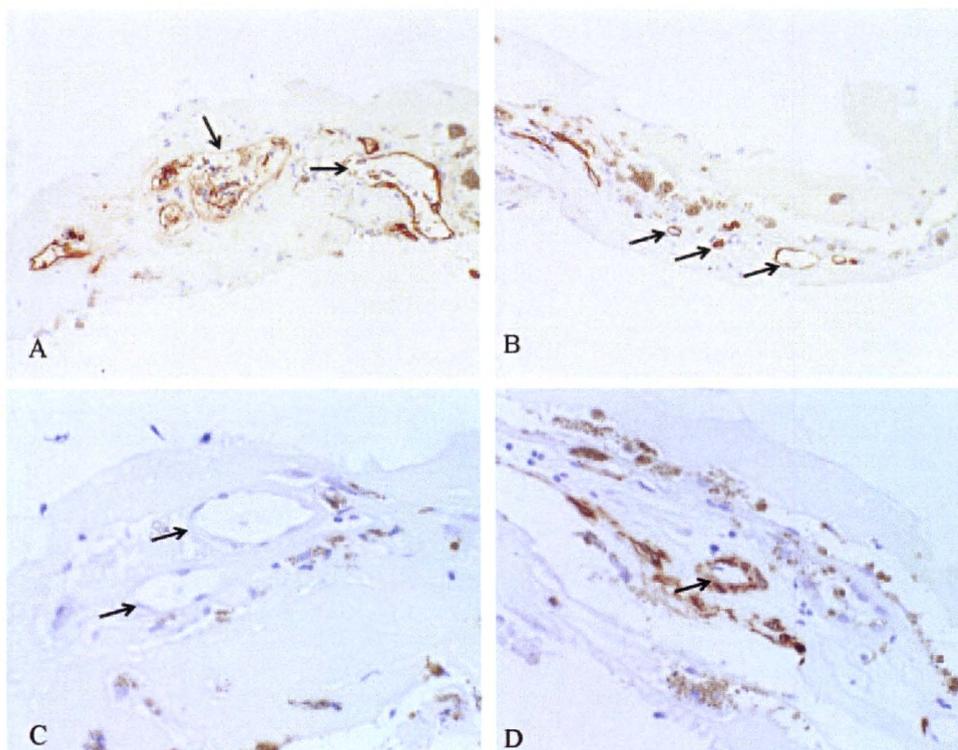


FIGURE 6. Immunohistochemical comparison between the PCV (A, C) and CNV (B, D) portions. (A) Vascular endothelium expressing the CD34 marker in the PCV portion. Note the discontinuous endothelium stained with CD34 (*arrow*). (B) Vascular endothelium expressing the CD34 marker in the CNV portion. Note the continuous endothelium stained with CD34 (*arrow*). (C) α -SMA-positive cells were absent in the hyalinized vessels located on the PCV portion. (D) α -Smooth muscle actin-positive pericytes are present in vascular channels located on the CNV portion. Magnification: (A, B) $\times 50$; (C, D) $\times 100$.

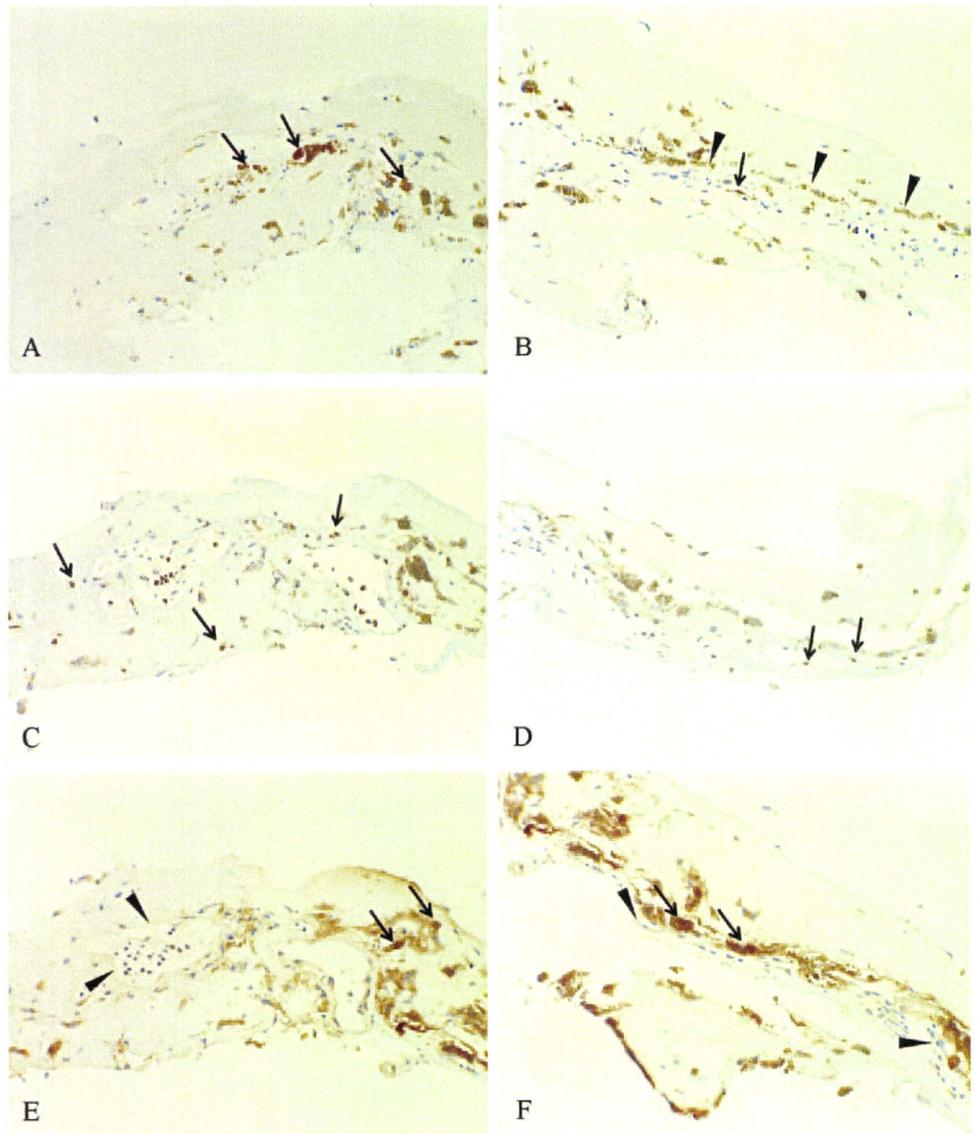


FIGURE 7. Immunohistochemical comparison between the PCV (A, C, D) and CNV (B, D, F) portions. (A) Macrophages expressing the CD68 marker. Note the presence of CD68-positive foamy macrophages around the dilated vascular cavity in the PCV portion. (B) Macrophages expressing the CD68 marker. A few CD68-positive cells are present in the stroma of the CNV portion (arrow). Arrowhead: RPE. (C, D) HIF-1 α positive mononuclear cells are located in the stroma of specimens in both lesions (arrow). (E) Immunoreactive deposits of VEGF are present at macrophages (arrow), but not at vascular endothelial cells in the PCV portion (arrowhead). (F) Immunoreactive deposits of VEGF are present at macrophages and RPE cells (arrow), but not at vascular endothelium cells in the CNV portion (arrowhead). Original magnification: (A-F) $\times 50$.

showed granulation tissue proliferation, supporting the concept that CNV represents a stereotypic, nonspecific wound repair response.³⁰⁻³⁵ In contrast, little if any fibrosis or granulation tissue proliferation was observed in the PCV portion.

The histopathologic characteristics of PCV include hyalinization of vessels, extravasation of plasma protein and deposition of basement membrane-like material. Hyalinized vessels in PCV were negative for α -SMA expression, although pericytes of vessels in CNV were immunoreactive for α -SMA. This finding may indicate that smooth muscle cells of choroidal vessels in PCV had disappeared due to the increased intraluminal pressure resulting from systemic hypertension. Furthermore, Kondo et al.³⁶ reported that the elastin gene, which is a potent and specific regulator of the migration and proliferation of vascular smooth muscle cells, is a susceptibility gene for PCV. Therefore, elastin gene dysfunction may accelerate the disappearance of the smooth muscle cells of choroidal vessels in PCV.

Immunoreactive expressions of CD34 revealed discontinuity in the vascular endothelium in the PCV portion. This finding suggests that eddy diffusion of blood flow in hyalinized and dilated vessels causes sufficient damage to the vascular endothelium to cause sloughing. Recent investigations have demon-

strated VEGF also to be expressed in vascular endothelial cells and RPE cells of surgically excised subfoveal fibrovascular tissues of human CNV tissue.^{31,35} In our study of PCV, VEGF positivity was recognized in macrophages, fibroblast-like cells and RPE cells, but not in vascular endothelial cells. Tong et al.³⁷ reported that aqueous levels of VEGF in eyes with PCV were significantly lower than those in eyes with exudative AMD. These findings raise the possibility that these are distinct clinical entities with different pathogenic processes, and that VEGF may hardly contribute to the occurrence of PCV.

It was also reported that eyes with PCV sometimes had the appearance of classic CNV on FA and, as a result, the condition was wrongly attributed to type 2 CNV or to pure fibrinous tissue without CNV.^{20,38} In our study, three of the five eyes showed discontinuity in the RPE, and PCV portions including hyalinized vessels were found above the level of the RPE in two of the five cases. We speculated that the elevation of choroidal vessels through RPE breaks and the vulnerable Bruch's membrane, as in our cases 2 and 3, may be attributable to rising intratissue pressure caused by massive exudation from hyalinized vessels, which would result in a classic CNV pattern on FA.

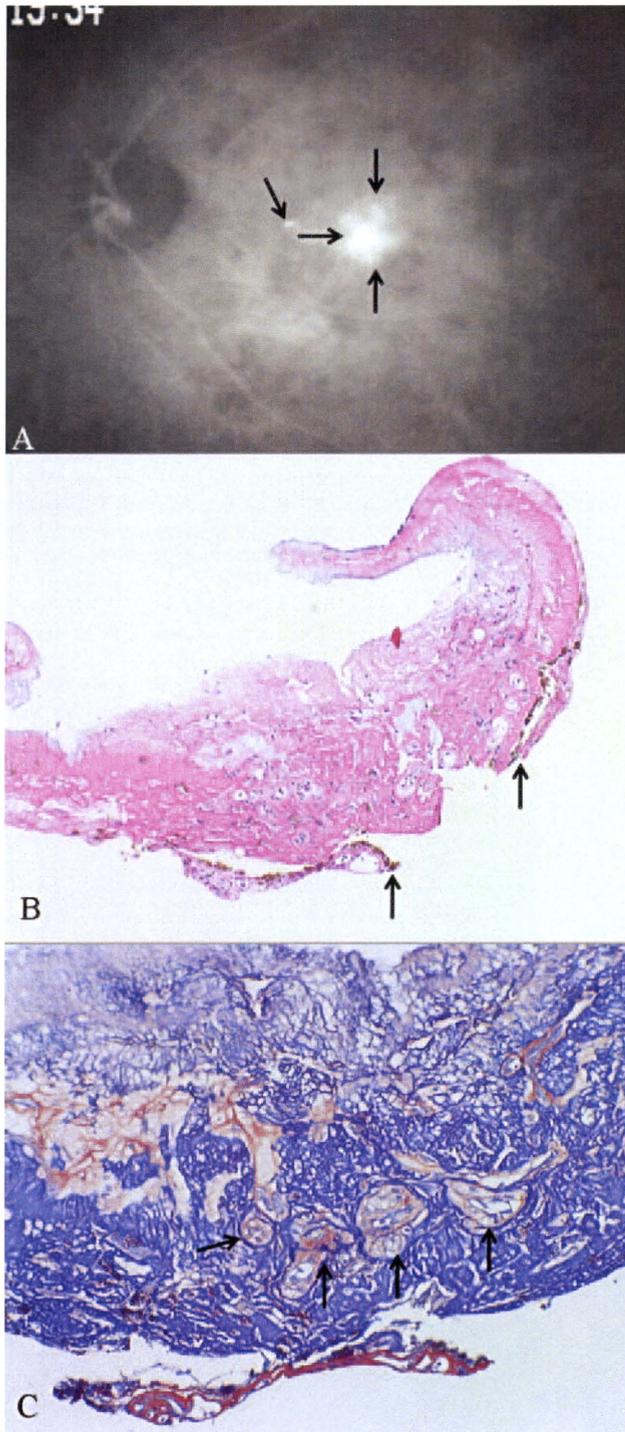


FIGURE 8. (A) IGA showing a polyplike dilatation at the macula (arrow). (B) Many hyalinized vessels with exudative change were seen above the RPE. A break in the RPE was documented in this specimen (arrow) (hematoxylin-eosin). (C) Hyalinization of vessels with exudative change around the vessels (arrow). The fibrovascular tissue underneath the RPE, stained red by PTAH, was thought to be occult CNV (phosphotungstic acid hematoxylin). Magnification, (B) $\times 25$; (C) $\times 50$.

However, we cannot rule out the possibility that PCV and CNV can occasionally exist in the same eye simultaneously. Thus, there are two possibilities. The first is that PCV lesion and CNV associated with AMD are likely present together incidentally. The second possibility is that CNV may grow

secondarily as a result of a wound repair reaction to a collapse of the RPE or Bruch's membrane in advanced PCV. Furthermore, positivity for HIF-1 α was observed in the nuclei of lymphocyte-like cells in the PCV. HIF-1 α has been recognized as a transcription factor induced by hypoxia, and exposure of the cells to hypoxia (1% O₂) reportedly induced nuclear translocation of HIF-1 α from the cytoplasm.³⁹ It has also been shown to stimulate transcriptions of multiple genes that are upregulated by hypoxia, including VEGF.⁴⁰ Thus, the environment in PCV specimens is thought to be hypoxic, and HIF-1 α may accelerate the expression of VEGF which would stimulate CNV formation.

Yuzawa et al.⁷ have reported polypoidal lesions sometimes containing many vessels in various abnormal configurations shown on IGA with confocal scanning laser ophthalmoscopy. The present clinicopathologic study has demonstrated that the presence of many vessels in case 3 corroborates these IGA findings.

In this study, PCV was characterized by vascular hyalinization followed by massive extravasation of plasma protein, deposition of basement membrane-like material, and lack of granulation tissue proliferation. To our knowledge, hyalinization of CNV has not been reported. Furthermore, dilated hyalinized vessels were observed beneath but not within Bruch's membrane in our case 1. These findings demonstrate that hyalinized vessels are the choroidal vasculature with arteriosclerotic changes similar to the hyalinization seen in other parts of the body (i.e., they do not represent neovascularization). Furthermore, massive exudation of fibrin and blood plasma from dilated hyalinized choroidal vessels may raise choroidal tissue pressure sufficiently to produce protrusion of choroidal tissues through the weakened or disrupted RPE and Bruch's membrane.

The results in this study suggest that hyalinization of choroidal vessels is characteristic of PCV and that arteriosclerosis is an important pathologic feature.

Acknowledgments

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