TABLE 3. Replication Study Using Cataract Patients

		Cataract Patients (n = 336)		vs. tAMD (n = 455)		vs. PCV (n = 579)	
		Minor Allele	MAF	P*	OR (95% CI)*	P*	OR (95% CI)*
C2	rs547154	T	0.092	0.0028	0.51 (0.33–0.79)	0.0038	0.56 (0.38–0.83)
CFB	rs541862	C	0.091	0.0028	0.51 (0.33–0.79)	0.0046	0.57 (0.38–0.84)

MAF, minor allele frequency in the cataract patients.
* Adjusted for age and sex.

(rs547154: P = 0.018, odds ratio [OR] = 0.57, 95% confidence interval [CI] = 0.35–0.91; rs541862: P = 0.016, OR = 0.56, 95% CI = 0.35–0.90) and PCV (rs547154: P = 0.0062, OR = 0.54, 95% CI = 0.35–0.84; rs541862: P = 0.0061, OR = 0.54, 95% CI = 0.35–0.84). These associations remained significant, even after a permutation procedure for multiple test correction (corrected $P < 0.05$). There was no SNP in the four tested SNPs across the *C2/CFB* locus, which showed a significant difference between tAMD and PCV.

Because the age of the controls was definitely younger than that of the cases, to adjust for a birth cohort effect, differential survival, or survivorship, we also performed a stratification analysis using 420 controls aged 50 years or older. This stratified cohort included 212 (50.5%) men, 208 (49.5%) women, 223 (53.3%) never smokers, and 195 (46.7%) ever smokers. The mean age ± SD of the group was 62.68 ± 7.67 years. We found that the associations of *C2* rs547154 and *CFB* rs541862 remained statistically significant in both tAMD (rs547154, P = 0.0048; rs541862, P = 0.0042) and PCV (rs547154, P = 0.0076; rs541862, P = 0.0075) in this stratification analysis, as well.

Next, we confirmed the positive associations using a second control cohort of 336 elderly cataract patients. The mean age ± SD of the cataract patients was 74.16 ± 8.42 years (range, 43–94), and 142 (42.3%) male and 194 (57.7%) female patients were included. Table 3 shows the result of this replication analysis. Significant associations for developing both tAMD and PCV with *C2* rs547154 and *CFB* rs541862 were also shown in this evaluation ($P < 0.05$). In addition, we performed haplotype analysis using the cataract controls (Table 4). Haplotype analysis revealed that a common haplotype across the *C2/CFB* locus conferred a significant risk for both tAMD and PCV (P = 0.0030 and 0.0001, respectively) and a rare haplotype protectively associated with both tAMD and PCV (P = 0.0001 and 0.0016, respectively).

Finally, we conducted a logistic regression analysis that included the effects of the most robust Japanese AMD/PCV-associated variants, *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292), as well as age, sex, and smoking status. Because *C2* rs547154 and *CFB* rs541862 were in strong LD (pair-wise D′ = 1.0 and r^2 = 1.0), we analyzed rs547154 as the representative SNP of the *C2/CFB* locus. Table 5 shows the result of this logistic regression analysis. *C2/CFB* rs547154 remained significant both in tAMD and PCV, even after including the effects of these covariates (vs. tAMD: P = 0.0073, OR = 0.47, 95% CI = 0.27–0.82; vs. PCV: P = 0.0083, OR = 0.53, 95% CI = 0.33–0.85). After considering the effects of three major AMD-associated loci, we found that the effect of smoking was diminished in the risk for PCV (P = 0.292), and just a marginal association was found for tAMD (P = 0.0693).

DISCUSSION

The present study shows the significance of polymorphisms in *C2* and *CFB* for development of tAMD and PCV in a relatively large sample of Japanese patients. As a result of comparing the genotypic distributions of *C2/CFB* variants in a sample of Japanese patients with tAMD (n = 455) or PCV (n = 581) and in two independent control groups (865 healthy Japanese individuals and 336 cataract patients), we found that *C2* rs547154 (IVS10) and *CFB* rs541862 showed significant associations with the risk for both tAMD and PCV, with protective effects against the risk of the diseases.

Replication is the gold standard for assessing statistical results from genetic studies. However, a real result may fail to be replicated for numerous reasons, including inadequate sample size or variability in phenotype definitions across independent samples.[34] Although numerous reports have shown a significant association between *C2/CFB* variants and AMD in Caucasians, all studies in Asians have been unable to replicate these results.[16,17] However, in the present study, we clearly showed a significant association of *C2/CFB* variants for developing AMD in a Japanese cohort. Considering that minor allele frequency is similar between our cohort and previous reports, this discrepancy would be due to the small sample size used in the previous reports on Asian cohorts. Our study indicates that previous studies on the same subject did not reach statistical significance, and that large cohorts are needed to have enough statistical power to detect the association of the *C2/CFB* locus.

To date, all reports on Asian cohorts have shown a lack of association between *C2/CFB* polymorphism and PCV.[26,27] However, recently, Lima et al.[28] showed a positive association with PCV in Caucasians, even though incidence of PCV is lower in Caucasians than in Asians. In the present study,

TABLE 4. Distribution of Haplotypes and the Results of the Association Tests

Haplotype				Frequencies		P		
rs547154	rs541862	rs2072633	rs4151672	tAMD	PCV	Cataract Controls	Cataract vs. tAMD	Cataract vs. PCV
G	T	G	C	0.55	0.57	0.47	0.0030	0.0001
G	T	A	C	0.39	0.36	0.41	0.527	0.0467
T	C	A	C	0.044	0.053	0.092	0.0001	0.0016
G	T	G	T	0.018	0.022	0.029	0.109	0.264

tAMD, typical age-related macular degeneration.

TABLE 5. Comparison of the Significance of Major AMD-Associated Factors

	tAMD		PCV	
	P*	OR (95% CI)	P*	OR (95% CI)
Age, y	<0.0001	1.21 (1.18-1.24)	<0.0001	1.18 (1.15-1.20)
Sex, women/men	0.0127	1.87 (1.14-3.07)	<0.0001	2.64 (1.72-4.04)
ARMS2/HTRA1 rs10490924 (G/T)	<0.0001	2.43 (1.85-3.20)	<0.0001	2.02 (1.60-2.56)
CFH rs800292 (A/G)	<0.0001	1.89 (1.41-2.55)	<0.0001	2.02 (1.57-2.60)
C2/CFB rs547154 (G/T)	0.0073	0.47 (0.27-0.82)	0.0083	0.53 (0.33-0.85)
Smoking, never/ever	0.0693	1.56 (0.97-2.52)	0.292	1.25 (0.83-1.87)

* A logistic regression model was used to analyze the association between covariates.

C2/CFB variants were clearly associated with PCV. Our result is therefore in agreement with that of the Caucasian cohort used by Lima et al. Hence, our study also supports that there is no difference between tAMD and PCV in the role of *C2/CFB* for development of the disease.

In addition, we found the association of *C2/CFB* variants was unchanged, even when we adjusted for the effects of other established risk factors for AMD (age, sex, smoking, and a genetic background including *ARMS2* A69S and *CFH I62V*). In this study, common genetic variations at all three loci were associated with PCV, similar to that already documented in AMD—that is, SNPs that conferred a higher risk or protection from the disease in AMD were associated with the same in PCV. Furthermore, logistic regression analysis revealed that the role of environmental factors (smoking) diminishes when the effects of the three major AMD-associated loci (*ARMS2/HTRA1*, *CFH*, and *C2/CFB*) are taken into consideration. This result indicates that genetic factors have an enormous influence on whether people develop AMD and/or PCV. Among all covariates, *ARMS2/HTRA1* variants had the largest effect on the risk for tAMD (OR = 2.43), whereas sex had the largest effect on development of PCV (OR = 2.64). In a previous meta-analysis study,[35] the prevalence of late AMD in Asian women was reported to be much lower than in Asian men; on the other hand it is said that those with PCV are predominately male.[20] Considering the high prevalence of PCV in Asian populations, these results suggest that men would be more likely to develop PCV. In our study, genetic factors had important roles in the development of both tAMD and PCV. Thus, our results indicate that differences in sex would affect phenotypic differences in AMD.

In the present study, we evaluated different SNPs from those examined in the original study,[8] because minor allele frequencies of the SNPs evaluated in Caucasians were extremely low in the Japanese. To confirm the association reported in Caucasians, we also genotyped *C2* rs9332739, reported to be positive in the original study in cataract controls. However, there was no significant association in *C2* rs9332739 for development of tAMD and PCV in our cohort, because of its low allele frequency (data not shown), and *C2* rs9332739 did not have an impact on the result of haplotype analysis

We also grouped the current smokers and the former smokers into ever smokers, because this group had the highest tendency to develop PCV.[31] However, smoking was not found to have a significant independent association with the development of either tAMD or PCV in this study. Considering that smoking status was obviously different between the cases and the controls, this association should reach statistical significance if the number of participants were increased. Another reason for the lack of association could be because of the heterogeneity of smoking status. As with the general trend, the former smokers were older than others, and more men than women had smoked in our cohort (data not shown). In addition, we could not exclude the possibility that there were interactions between genetic and environmental factors or between genes; several studies have reported the presence of interactions between *ARMS2/HTRA1*, *CFH*, and smoking in AMD[9,10,36] and PCV.[31] Hence, further studies should be performed to ascertain the effects of interaction of different risk factors in the development of disease, including AMD-associated genes and smoking.

Another limitation of the present study is the difference between the case and control samples. The control samples were definitely younger than those in the case group, which means that some of these young controls may develop AMD or PCV in the future. To exclude a potential confounder of genetic background in age, we confirmed that our results were unchanged, even after a stratification analysis adjusting for the difference in age. In addition, to avoid a sampling error, we performed a replication study using another control group of a much closer age to the cases (cataract patients without ARM) and found a significant association between *C2/CFB* variants and development of AMD/PCV. However, because the prevalence of late AMD in the Japanese population is reported to be 0.5%,[37] the magnitude of the statistical bias of an association analysis should be negligible. In addition, considering that the case-control association analyses using such subjects are less apt to be statistically significant, our positive results should be acceptable.

Recently, subretinal drusenoid deposits, called reticular pseudodrusen, were differentiated from soft drusen with spectral domain optical coherence tomography (SD-OCT)[38] and were reported to be associated with late AMD.[39,40] We also evaluated whether *C2/CFB* variants are associated with developing reticular pseudodrusen in a small number of participants (n = 91) who had SD-OCT and autofluorescence imaging. However, we could not find a significant association between *C2/CFB* variants and the incidence of reticular pseudodrusen (data not shown). Further studies are needed to ascertain the association between *C2/CFB* variants and developing reticular pseudodrusen.

In conclusion, this study provides the first evidence that *C2/CFB* variants play a role in the risk of both neovascular AMD and PCV in Asians. Inflammation plays a central role in the pathobiology of AMD, with *C2* and *CFB* both encoding regulatory proteins that activate the complement pathway. As the inhibition of CFB with a specific chemical binding entity has been suggested to be a viable approach for the treatment of neovascular AMD,[41] our findings may suggest the potential effectiveness of such treatments by using anti-inflammatory agents, not only for AMD but also for PCV.

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Association of Elastin Gene Polymorphism to Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy

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Purpose. To see if there is an association in Japanese between elastin gene (*ELN*) polymorphisms and neovascular age-related macular degeneration (AMD) or its subtypes, typical AMD (tAMD) and polypoidal choroidal vasculopathy (PCV).

Methods. The authors genotyped five single nucleotide polymorphisms (SNPs), rs2301995, rs2856728, rs868005, rs884843, and rs13239907, at Kyoto University and Saitama Medical University. A case-control study was performed on 1296 patients with AMD and 478 controls.

Results. A statistically significant association was detected between the rs2301995 SNP and AMD ($P = 0.018$). Furthermore, subtype analysis revealed a significant association of rs2301995 with tAMD ($P = 0.0018$), but not with PCV. The genotype distribution of rs2301995 also differed significantly between tAMD and PCV ($P = 0.00030$). The trend in genotype distribution of rs2301995 was similar between the Kyoto and the Saitama studies. The A allele frequency was higher in tAMD, whereas it was similar in PCV and in controls, which is opposite to that reported in a previous study that the A allele frequency is higher in PCV, whereas it is similar in tAMD and in controls. Haplotype analysis also showed that the *ELN* polymorphism is significantly associated with tAMD ($P = 0.0055$), but not with PCV.

Conclusions. *ELN* is associated with AMD in Japanese. Furthermore, the findings suggest that *ELN* is a susceptibility gene for tAMD but not for PCV, which is opposite to that reported in a previous study that *ELN* is the susceptibility gene for PCV but not for tAMD. (*Invest Ophthalmol Vis Sci.* 2011;52:8780-8784) DOI:10.1167/iovs.11-8205

Neovascular age-related macular degeneration (AMD) is divided into polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation, and typical age-related macular degeneration (tAMD) that includes predominantly classic choroidal neovascularization (CNV) type, minimally classic CNV type, and occult with no classic CNV type. Differences between tAMD and PCV have been investigated for more than a decade. Although PCV is regarded as a subtype of AMD, its prognosis has been reported to be better than that of tAMD.[1,2] Furthermore, the response to treatment differs between tAMD and PCV. It has been shown that photodynamic therapy is more effective for PCV than that for tAMD.[3-5] In contrast, anti-vascular endothelium growth factor (VEGF) treatment just barely inactivates the polypoidal lesions of PCV, although it works well on the CNV associated with tAMD.[6-9] The prevalence of PCV and tAMD also differs depending on ethnicity. In Caucasians, PCV is seen in only 8-13% of neovascular AMD,[2] whereas it has been shown that 41.3-54.7% of neovascular AMD in Japanese patients have PCV.[10,11] These differences suggest genetic background differences in the development of tAMD and PCV.

Susceptibility genes for AMD have been investigated intensely, and evidence has shown that the *CFH* gene and the *ARMS2/HTRA1* gene are associated with AMD, tAMD, and PCV.[12-18] We have recently shown that the association of the *ARMS2* gene is stronger for tAMD than for PCV, whereas the association of the *CFH* gene is similar between tAMD and PCV.[19] Discovery of a susceptibility gene associated only with tAMD but not with PCV, or vice versa, would deepen our understanding of the difference in pathogenesis between tAMD and PCV.

Kondo and colleagues[20] evaluated five tag SNPs of the elastin gene (*ELN*) in Japanese, and showed that its polymorphism is associated with PCV but not with tAMD. Although they discussed the possibility that they might have underestimated the association with tAMD because of a type I error due to low samples sizes ($n = 285$), *ELN* might explain the different mechanisms involved in the development of tAMD and PCV. In Caucasians, however, it has been shown that *ELN* is not associated with PCV or AMD.[21,22]

Bruch's membrane is an elastin-rich extracellular matrix between retinal pigment epithelium and choroidal capillaries, whose defect can lead to CNV formation. Elastin polymerization deficit has been shown to lead to larger CNV in laser-

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TABLE 1. Characteristics of Study Population

	Kyoto			Saitama		
Factor	**Controls**	**tAMD**	**PCV**	**Controls**	**tAMD**	**PCV**
Number	336	408	518	142	216	154
Mean age ± SD, y	74.2 ± 8.4*	77.7 ± 8.4*	75.1 ± 8.5*	68.4 ± 9.8†	72.7 ± 8.7†	71.8 ± 7.8†
Sex, male/female	142/194‡	293/115‡	381/137‡	74/68‡	158/58‡	122/32‡

* $P < 0.0032$ each other with the Scheffé post hoc test.
† $P < 0.00031$ each other with the Scheffé post hoc test, except for tAMD and PCV ($P = 0.59$).
‡ $P < 0.0001$ with χ^2-test.

induced CNV in mouse.[23] Furthermore, histophathologic studies showed disruption of the elastic layer of polypoidal vessels in PCV. Evaluation of elastin in CNV development would lead to more precise understanding of the difference between tAMD and PCV.[24,25] In the present study we evaluated the five tag SNPs of *ELN* at Kyoto University and, separately, at Saitama University, using the same probes to examine the association of *ELN* to AMD and to its subtypes tAMD and PCV in a relatively large cohort ($n = 1774$).

Materials and Methods

This study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board/Ethics Committee of Kyoto Graduate School of Medicine, Kyoto, Japan, and by the Ethics Committee of Saitama Medical University, Saitama, Japan. Written informed consent was obtained from each patient.

In all, 926 patients with neovascular AMD and 336 patients with cataract but no AMD selected as control were recruited from the Department of Ophthalmology at Kyoto University Hospital and from the Ophthalmology Department at Fukushima Medical University Hospital. Of the 926 patients, 408 had tAMD and 518 had PCV. A total of 142 control subjects and 370 patients with neovascular AMD, including 216 with tAMD and 154 with PCV, were recruited from Saitama Medical University Hospital. All subjects in the present study were unrelated and all were of Japanese descent. The diagnosis of PCV was based on indocyanine green angiography, which showed a branching vascular network that terminated in polypoidal swelling. Typical AMD showed classic CNV, occult CNV, or both. All diagnoses in the Kyoto study and the Saitama study were made by three retina specialists at each facility using fundus photograph, fluorescein and indocyanine green angiography, and optical coherence tomography; a fourth specialist was called on when the subtype classification could not be decided on by the initial three reviewers. Less than 5% of the diagnosis was made with the help of the fourth specialist.

Genomic DNAs were prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan, or Wizard Genomic DNA Purification Kit; Promega, Madison, WI). Tag SNPs were selected by use of a commercial database (HapMap database phase 2 release 22) by the tagger pairwise method with an R^2 cutoff of 0.8 and minor allele frequency (MAF) cutoff of 0.2, which included the five SNPs (rs2301995, rs2856728, rs868005, rs884843, and rs13239907) that were previously examined,[20,21] and were genotyped using an SNP assay (TaqMan, with the ABI PRISM 7700 system or 7000 system; Applied Biosystems, Foster City, CA). Mean ages were compared using ANOVA with post hoc comparisons tested by the Scheffé procedure. Allele frequency and sex ratio were compared with the χ^2 test and the genotype distribution was compared with the χ^2 test for trend or its exact counterpart. Age and sex differences were adjusted with logistic regression analysis software (R software, provided in the public domain by the R Foundation for Statistical Computing, Vienna, Austria, available at http://www.r-project.org/) by fitting the number of minor allele carried as an ordinal covariate, and age and sex as continuous and categorical covariates. Values of $P < 0.05$ were considered to be statistically significant.

Results

Demographics of the study population are shown in Table 1. The mean ages and sex ratios were significantly different between controls and cases at each facility. Table 2 shows the genotype distributions of rs2301995, rs2856728, rs868005, rs884843, and rs13239907 in both the Kyoto study and the Saitama study. Since the genotype frequencies were not significantly different between the Kyoto study and the Saitama study, we pooled the data to evaluate the association of the five SNPs to AMD, tAMD, and PCV.

A statistically significant association was detected between rs2301995 and AMD ($P = 0.018$; Table 3). When the subtypes of AMD were evaluated, rs2301995 was significantly associated with tAMD ($P = 0.0018$), with the A allele as a risk, but was not associated with PCV. In both the Kyoto and the Saitama studies, the frequencies of the minor allele of rs2301995 and rs2856728 were higher in tAMD, whereas they were similar in controls and in PCV. The genotype distributions of rs2301995 and rs2856728 were also significantly different between tAMD and PCV ($P = 0.00030$ and 0.00012).

TABLE 2. Genotype Distribution of *ELN* Gene SNPs in Control Subjects and in Patients with AMD, tAMD, or PCV in Kyoto and Saitama Studies

Study/Factor	rs2301995 AA/AG/GG	rs2856728 CC/CT/TT	rs868005 CC/CT/TT	rs884843 GG/GA/AA	rs13239907 GG/GA/AA
Kyoto					
Controls, *n* (%)	13/99/217 (4/30/66)	25/125/182 (8/38/54)	22/107/203 (7/32/61)	58/167/108 (17/51/32)	133/161/38 (40/49/11)
AMD, *n* (%)	50/307/558 (5/34/61)	87/341/479 (10/38/52)	38/324/555 (4/35/61)	170/478/262 (19/52/29)	389/407/118 (43/44/13)
tAMD, *n* (%)	28/148/227 (7/37/56)	44/157/198 (11/39/50)	15/136/255 (4/33/63)	80/216/105 (20/54/26)	171/183/48 (43/45/12)
PCV, *n* (%)	22/159/331 (4/31/65)	43/184/281 (8/36/56)	23/188/300 (45/37/59)	90/262/157 (18/51/31)	218/224/70 (43/43/14)
Saitama					
Controls, *n* (%)	5/37/100 (4/26/70)	10/47/82 (7/34/59)	5/28/63 (5/30/65)	17/76/45 (12/55/33)	55/70/15 (39/50/11)
AMD, *n* (%)	16/105/234 (5/30/65)	26/118/196 (8/35/57)	11/29/51 (12/32/56)	68/141/125 (20/42/38)	151/160/43 (43/45/12)
tAMD, *n* (%)	12/58/134 (6/28/66)	20/65/108 (10/34/56)	9/13/33 (16/24/60)	41/74/71 (22/40/38)	87/90/26 (43/44/13)
PCV, *n* (%)	4/47/100 (3/31/66)	6/53/88 (4/36/60)	2/16/18 (6/44/50)	27/67/54 (18/46/36)	64/70/17 (42/47/11)

TABLE 3. Association of rs2301995 and rs2856728 to AMD, tAMD, and PCV versus Controls

	Allele		Genotype		
SNP/Factor	*P* Value	OR (95% CI)	*P* Value	Adjusted *P**	Minor Allele
rs2301995					
AMD	0.041	1.22 (1.01–1.48)	0.044	0.018	A
tAMD	0.0028	1.38 (1.12–1.71)	0.0036	0.0018	
PCV	0.47	1.12 (0.73–1.72)	0.48	0.33	
rs2856728					
AMD	0.77	1.05 (0.76–1.45)	0.78	0.28	C
tAMD	0.37	1.18 (0.83–1.68)	0.40	0.089	
PCV	0.57	0.89 (0.61–1.32)	0.57	0.89	
rs868005					
AMD	0.86	0.98 (0.81–1.19)	0.86	0.58	C
tAMD	0.72	1.15 (0.89–1.47)	0.72	0.67	
PCV	0.55	0.99 (0.79–1.25)	0.55	0.50	
rs884843					
AMD	0.23	0.91 (0.78–1.08)	0.22	0.42	G
tAMD	0.098	0.86 (0.73–1.03)	0.094	0.11	
PCV	0.59	0.95 (0.81–1.173)	0.59	0.88	
rs13239907					
AMD	0.72	1.03 (0.88–1.20)	0.72	0.60	G
tAMD	0.66	1.04 (0.87–1.24)	0.66	0.51	
PCV	0.84	1.01 (0.85–1.21)	0.84	0.98	

OR, odds ratio; CI, confidence interval.
* Adjusted for age and sex with regression analysis.

Similarly to Kondo et al.,[20] we performed a haplotype analysis (Haploview) using three SNPs: rs868005, rs884843, and rs2301995. The estimated frequencies are shown in Table 4. A significant difference was noted for the T-G-A in an analysis between controls and tAMD ($P = 0.0055$) and also in an analysis between tAMD and PCV ($P = 0.014$).

DISCUSSION

In the study reported herein, we showed that the *ELN* polymorphism is associated significantly with AMD, and subtype analysis revealed that *ELN* is a susceptibility gene for tAMD but not for PCV. Our findings are consistent with those of a previous report by Kondo et al.[20] in that *ELN* is a susceptibility gene for AMD. However, in that report they showed that the *ELN* polymorphism is associated with PCV, but not with tAMD. Furthermore, they reported that MAFs of rs2301995 and rs2856728 were higher in PCV, whereas they were similar in controls and in tAMD (Table 5). This is opposite to our findings—that the MAFs of rs2301995 and rs2856728 are higher in tAMD and are similar in controls and in PCV. The genotype distributions of rs2301995 and rs2856728 had a similar trend between the Kyoto and Saitama studies. Although further investigation is needed, the consistent trend in genotype distribution in the Kyoto study and in the Saitama study suggests that *ELN* is a susceptibility gene for tAMD, but not for PCV.

In Caucasians, Lima et al.[21] evaluated the *ELN* rs2301995 polymorphism in 66 patients with PCV and in 368 unaffected controls, and showed that it was not associated with PCV. Ennis et al.[22] also evaluated three *ELN* SNPs, rs868005, rs2071307, and rs11770302, in 479 AMD patients and in 479 controls, and showed that *ELN* is not associated with AMD in Caucasians. According to the report by Lima et al.,[21] the MAFs of the rs2301995 SNP in Caucasians were 6.3% in PCV, 5.4% in AMD, and 7.1% in controls, which are relatively lower than the MAFs in Japanese, which are reported to be 14–17%. Evaluating associations of SNPs whose MAFs are low tends to lead to false-negative results. For example, many East Asian studies reported that *CFH* Y402H SNP, the most significantly associated gene locus for Caucasian AMD, is not associated with AMD.[26–31] More recent studies, performed with a larger cohort or meta-analysis, however, revealed an association of the Y402H SNP to AMD, tAMD, and PCV in East Asians.[19,32] Further study with a larger cohort might be required to investigate the association of *ELN* rs2301995 SNP in Caucasians.

In the present study, haplotype analysis also supports the theory that *ELN* is associated with tAMD, not with PCV, in Japanese. The A-G-T haplotype was more prevalent in tAMD than that in controls and, furthermore, was significantly more prevalent in tAMD than that in PCV. However, Kondo and associates[20] reported that the A-G-T haplotype was more prevalent in PCV than that in controls or tAMD. The difference

TABLE 4. Haplotype Distribution of *ELN* Gene between Controls and tAMD, Controls and PCV, and tAMD and PCV

	Haplotype Frequency			*P* Value*		
Haplotype	Controls	tAMD	PCV	Controls vs. tAMD	Controls vs. PCV	tAMD vs. PCV
TAG	0.58	0.53	0.56	0.045	0.56	0.12
CGG	0.23	0.21	0.22	0.45	0.84	0.50
TGA	0.18	0.23	0.19	0.0055	0.54	0.014
TGG	0.015	0.017	0.018	0.70	0.62	0.92

* Corrected *P* value by permutation test (number of iterations = 1,000,000).

TABLE 5. Comparison of rs2301995 and rs2856728 Minor Allele Frequency

	rs2301995 A Allele Frequency				rs2856728 C Allele Frequency			
Factor	**Kyoto**	**Saitama**	**Pooled**	**Kondo et al.[20]**	**Kyoto**	**Saitama**	**Pooled**	**Kondo et al.[20]**
Controls	0.19	0.17	0.18	0.15	0.26	0.24	0.26	0.24
tAMD	0.25*	0.20	0.24*	0.14	0.31	0.27	0.30*	0.18
PCV	0.20	0.18	0.19	0.26†	0.27	0.22	0.26	0.32†

* $P < 0.001$ against controls and PCV.
† $P < 0.001$ against controls and tAMD.

between our findings and the previous report by Kondo et al.[20] might stem from selection bias or diagnosis difference among facilities. Furthermore, the association detected in our study and the previous study might be false-positive findings. In the present study, only one SNP of rs2301995 showed a significant association. When Bonferroni correction was applied, the *P* values of rs2301995 between control and tAMD become 0.042 in allele analysis and 0.027 in genotype analysis. The *P* value of the A-G-T haplotype between control and tAMD becomes 0.017. Further evaluation by other facilities would be indispensable.

The differences between tAMD and PCV in prognosis, reaction to treatments, and prevalence according to ethnicity suggest to us that the pathogenesis of tAMD and PCV is different, at least in part. Histopathologic studies have evaluated the pathologic features of PCV, and whereas some studies showed that the choroidal neovascularization of PCV is the same as that of tAMD, others showed that PCV consists of abnormalities of the inner choroidal vessels, and these differences have not led to a definitive understanding of the pathogenesis of these diseases.[24,25,33–38] Nakashizuka et al.[38] reported a lack of VEGF positivity in the vascular endothelial cells of PCV. In contrast, two other reports have shown the expression of VEGF in PCV[35,36] to be the same as that in CNV membranes secondary to tAMD.[39] Considered together with the relatively poor effects that anti-VEGF treatment have on PCV, *VEGF* gene polymorphism seems to be an attractive candidate in which to explore the difference between tAMD and PCV. However, evaluation of a previous report indicates no difference in *VEGF* gene polymorphism between tAMD and PCV.[11] Although the association difference of *ELN* to tAMD and PCV needs to be carefully judged from both the present study and previous studies, histopathologic studies of elastin expression in tAMD and PCV might reveal a difference in the pathogenesis between PCV and tAMD.

In summary, our findings indicate an association of the elastin gene variant with tAMD, but not with PCV, in Japanese subjects, but, since our findings are opposite those of previous studies, further investigation is warranted, and a genetic study may well deepen our understanding of the pathogenesis of tAMD and PCV.

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Treatment of Polypoidal Choroidal Vasculopathy With Photodynamic Therapy Combined With Intravitreal Injections of Ranibizumab

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• PURPOSE: To evaluate the 1-year efficacy and safety of photodynamic therapy (PDT) combined with intravitreal injections of ranibizumab for polypoidal choroidal vasculopathy (PCV).

• DESIGN: Retrospective chart review.

• METHODS: We retrospectively reviewed the medical records of 63 consecutive patients (66 eyes) with subfoveal PCV who were treated with PDT combined with intravitreal injections of ranibizumab. Of the 66 eyes, 29 had no history of treatment for PCV, 10 had been treated previously with only intravitreal injections of anti–vascular endothelial growth factor agents, and 27 had been treated previously with PDT. All eyes had a minimal follow-up of 12 months.

• RESULTS: The combined therapy reduced substantially the exudative change immediately after initiation of treatment. In treatment-naïve eyes, mean VA before treatment (0.47 ± 0.37 logarithm of the minimal angle of resolution [logMAR]) improved to 0.32 ± 0.30 ($P < .01$) at 3 months and to 0.29 ± 0.29 ($P < .01$) at 12 months. Polypoidal lesions were reduced in all eyes and disappeared completely in 79.1% of cases. In eyes treated previously with only anti–vascular endothelial growth factor therapy, some visual improvement was achieved, but in eyes treated previously with PDT, mean visual acuity (0.61 ± 0.45) deteriorated to 0.68 ± 0.52 at 12 months. Of all 66 eyes, 5 showed extensive postoperative subretinal hemorrhage, in 2 of which a vitreous hemorrhage developed, necessitating pars plana vitrectomy.

• CONCLUSIONS: PDT combined with ranibizumab led to significant visual recovery in treatment-naïve eyes with PCV, but not in eyes with PCV that had demonstrated recurrence after previous PDT. PDT in combination with ranibizumab still has a risk of the postoperative hemorrhagic complications. (Am J Ophthalmol 2012;153:68–80. © 2012 by Elsevier Inc. All rights reserved.)

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TODAY, INTRAVITREAL INJECTIONS OF ANTI-VASCULAR endothelial growth factor (VEGF) agents are a standard treatment for exudative age-related macular degeneration (AMD).[1,2] As a unique form of choroidal neovascularization (CNV),[3–5] polypoidal choroidal vasculopathy (PCV) is characterized by a branching vascular network that terminates in polypoidal lesions.[6–9] Similar to AMD,[10] recent findings of high concentrations of VEGF in aqueous humor[11] and high expression of VEGF in histologic specimens of PCV[12] have suggested that VFGF is involved also in the pathogenesis of PCV. In contrast to AMD, however, it has been reported that the treatment effects of anti-VEGF agents on the vascular lesions associated with PCV are limited.[13–24] In a recent report of PCV by Hikichi and associates, although exudative changes regressed with 3 monthly injections of ranibizumab, with concomitant improvement of visual acuity (VA), complete disappearance of the polypoidal lesions was achieved in only 26% of eyes.[25]

A number of studies have shown encouraging results of photodynamic therapy (PDT) on PCV.[26–30] In a report by Chan and associates, PDT led to complete regression of the polypoidal lesion in 95% of eyes with PCV and resulted in stable or improved VA 1 year after treatment.[27] Unfortunately, 1 or more years after successful treatment with PDT, some eyes have a recurrence of the PCV lesions and, consequently, a decrease in VA.[31,32] Akaza and associates reported that 64% of eyes with PCV that had been treated successfully with PDT showed a recurrence of the polypoidal lesions during a follow-up of 24 months. These recurrent polypoidal lesions often are accompanied by an exudative change, which results in a poor visual prognosis despite retreatment using PDT.[31–33]

Several investigators have reported promising short-term results of PDT combined with bevacizumab for the treatment of PCV.[34–38] Recently, Ruamviboonsuk and associates reported 1-year results of 12 eyes with PCV that were treated with PDT combined with ranibizumab.[39] To date, however, the efficacy and safety of this combined therapy has not been evaluated sufficiently.[37] In addition, most reports have involved PCV that had no previous treatments.[23,35,36,39] In the clinical setting, physicians often need to treat eyes in which PCV has recurred after initial treatment with PDT or eyes with chronic PCV that is refractory to anti-VEGF agents,[31–33] but, again,

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TABLE 1. Characteristics of Polypoidal Choroidal Vasculopathy in This Study Population

	Total	Treatment-Naïve Group	Anti-VEGF Group	PDT Group
No. of eyes	66	29	10	27
Age (years)	72.1 ± 8.0	70.7 ± 9.9	72.3 ± 5.5	73.5 ± 6.3
Gender (women/men)	20/46	11/18	2/8	7/20
No. of previous anti-VEGF treatments	—	—	4.3 ± 2.8	1.7 ± 1.0
No. of previous PDT treatments	—	—	—	1.6 ± 0.7
Initial visual acuity (logMAR)	0.53 ± 0.40	0.47 ± 0.37	0.51 ± 0.36	0.61 ± 0.45
Initial total foveal thickness (μm)	401.0 ± 188.9	390.3 ± 143.2	434.8 ± 243.2	399.3 ± 211.8
Cystoid macular edema	20	9	3	6
Serous retinal detachment	57	27	8	22
Subretinal hemorrhage	42	22	6	14
Pigment epithelial detachment	30	12	7	11
Greatest linear dimension	4530 ± 1339	4147 ± 1171	4968 ± 1704	4780 ± 1301
Additional anti-VEGF therapy	10	7	1	2
No. of additional anti-VEGF treatments	0.39 ± 1.20	0.69 ± 1.65	0.30 ± 0.95	0.11 ± 0.42
Range	1 to 7	1 to 7	1 to 3	1 to 2
Additional combined therapy	21	9	4	8
No. of additional combined therapy	0.38 ± 0.63	0.31 ± 0.47	0.50 ± 0.71	0.41 ± 0.75
Range	1 to 3	1	1 to 2	1 to 3

logMAR = logarithm of the minimum angle of resolution; PDT = photodynamic therapy; VEGF = vascular endothelial growth factor.

The treatment-naïve group consisted of eyes with no previous treatment for polypoidal choroidal vasculopathy (PCV). The anti-VEGF group consisted of eyes that were treated previously with only intravitreal injections of anti-VEGF agents for PCV with no history of PDT treatment. The PDT group consisted of eyes previously treated with PDT for PCV.

little information is available on the efficacy of combined therapy in such cases.[34] Accordingly, the study described herein was designed to study the safety and efficacy of PDT combined with ranibizumab for the treatment of symptomatic PCV, depending on the history of previous treatment(s).

METHODS

FOR THIS CASE SERIES, WE STUDIED RETROSPECTIVELY THE medical records of 66 consecutive eyes (63 patients) with subfoveal PCV that were treated with PDT combined with intravitreal injections of ranibizumab (Lucentis; Novartis, Bülach, Switzerland) at Kyoto University Hospital between the beginning of May 2009 and the end of February 2011. Inclusion criteria of the study were (1) symptomatic subfoveal PCV, (2) presence of exudative or hemorrhagic features involving the macula, (3) VA of 0.7 or less on a Landolt chart before treatment, and (4) a minimum follow-up of 12 months after the initial treatment. The diagnosis of PCV was based on indocyanine green angiography, which shows a branching vascular network that terminates in polypoidal swelling. In the current study, all eyes showed polypoidal lesions, a branching vascular network, or type 2 CNV beneath the foveal center. Eyes with other macular abnormalities (i.e., AMD, pathologic myopia, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, and other secondary CNV) were excluded from the study. However, pseudophakic eyes were included, but eyes with a history of vitrectomy were excluded.

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected VA with a Landolt chart, determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, spectral-domain optical coherence tomography ([OCT], Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and fluorescein and indocyanine green angiography (HRA-2; Heidelberg Engineering). Patients who had a visual disturbance resulting from PCV were offered PDT combined with an intravitreal injection of 0.5 mg ranibizumab. Injections of ranibizumab were performed in a sterile manner and prophylactic topical antibiotics were applied for 1 week after the injection. At 3 to 4 days after the intravitreal injection of ranibizumab, normal-fluence PDT was performed using a 689-nm diode laser unit (Visulas PDT system 690S; Carl Zeiss, Dublin, California, USA) after an injection of verteporfin (Visudyne; Novartis), according to PDT guidelines for AMD.[40] The greatest linear dimension chosen was based on fluorescein and indocyanine green angiograms, as previously described.[28] All polypoidal lesions and the entire branching vascular network detected with indocyanine green angiography and type 2 CNV detected with fluorescein angiography were included. Serous pigment

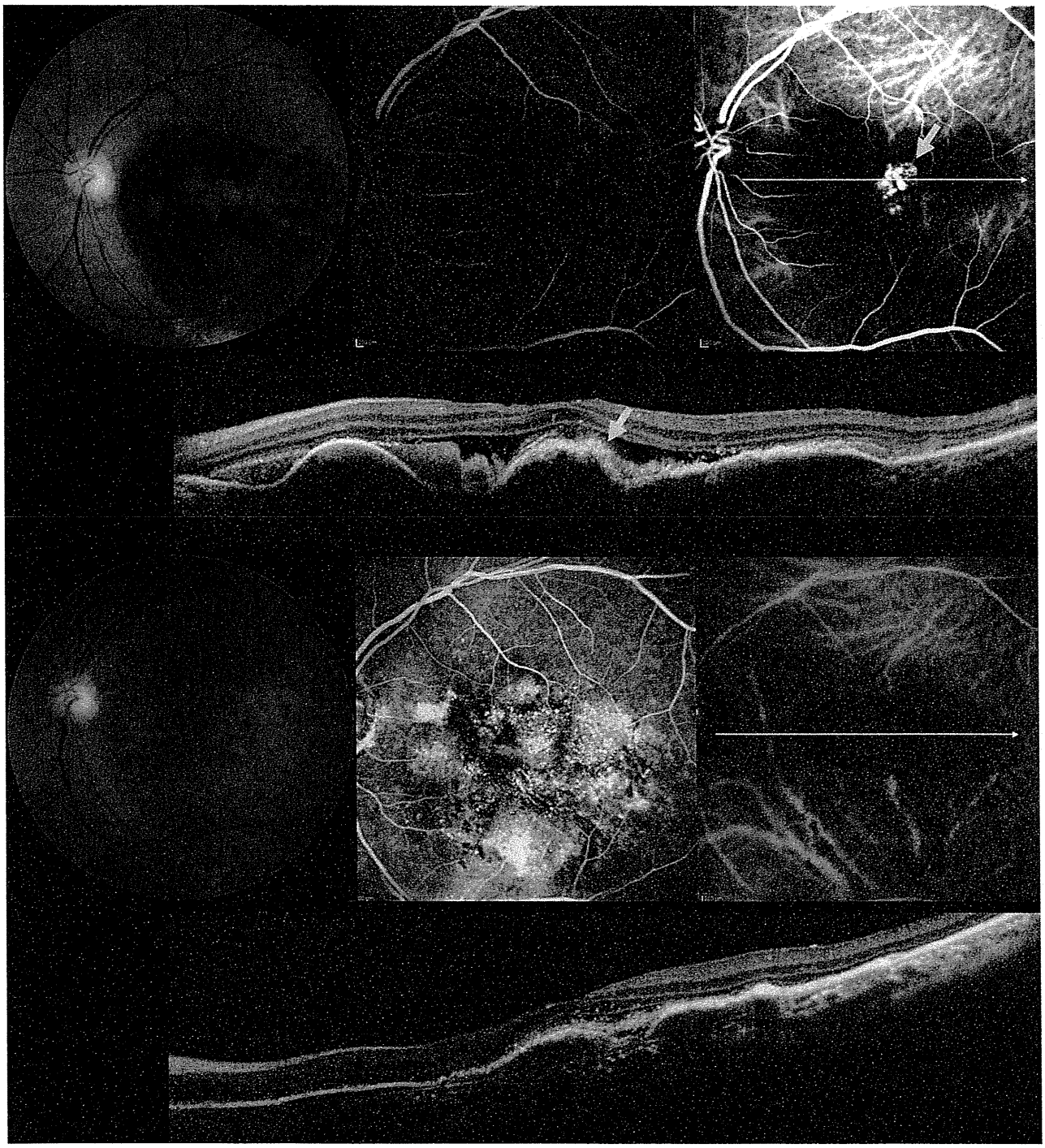

FIGURE 1. Treatment-naïve polypoidal choroidal vasculopathy. (Top left) Fundus photograph showing multiple serosanguineous pigment epithelial detachments with surrounding subretinal hemorrhage. Visual acuity of the left eye was 0.03. (Top middle) Fluorescein angiography image revealing occult choroidal neovascularization (CNV). (Top right) Indocyanine green angiography (ICGA) image showing polypoidal lesions that appear similar to a cluster of grapes (yellow arrow). (Second row) Section of an optical coherence tomography image along the white arrow shown in the Top right revealing a protrusion of retinal pigment epithelium resulting from polypoidal lesions (yellow arrow). Left eye was treated with photodynamic therapy combined with an intravitreal injection of ranibizumab. (Third row, left) Three months after the initial treatment, the subretinal hemorrhage has been absorbed and visual acuity of the left eye has improved to 0.3. (Third row, middle) Fluorescein angiography image showing atrophy of the retinal pigment epithelium. (Third row, right) ICGA image showing no polypoidal lesions. (Bottom) A section of optical coherence tomography along white arrow shown the Top right revealing minimal exudative change with smaller pigment epithelial detachments. Visual acuity at 12 months was 0.4 in the left eye.

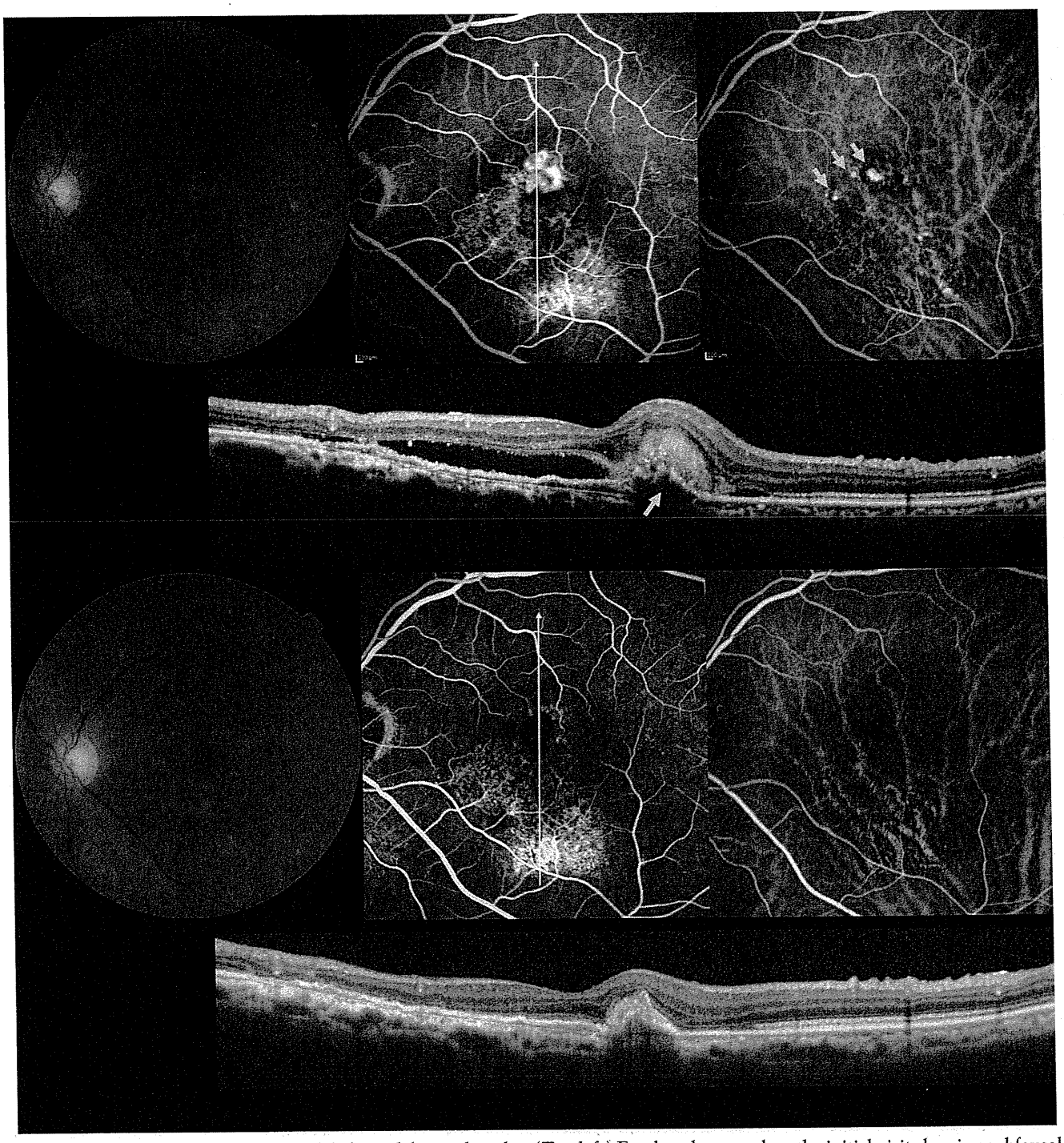

FIGURE 2. Treatment-naïve polypoidal choroidal vasculopathy. (Top left) Fundus photograph at the initial visit showing subfoveal exudates and hemorrhage. Visual acuity of the left eye was 0.4. (Top middle) Fluorescein angiography (FA) image revealing subfoveal classic choroidal neovascularization. (Top right) Indocyanine green angiography image showing a branching vascular network that terminates in polypoidal lesions (arrows). (Second row) Section of an optical coherence tomography image from along white arrow shown in the Top middle revealing a protrusion of retinal pigment epithelium resulting from polypoidal lesions (arrow) and overlying fibrin exudate. Left eye was treated with photodynamic therapy combined with an intravitreal injection of ranibizumab. (Third row, left) Three months after treatment, the subretinal hemorrhage and exudates have been absorbed and visual acuity of the left eye has improved to 0.8. (Third row, middle) FA image showing no classic choroidal neovascularization. (Third row, right) Indocyanine green angiography image showing no polypoidal lesions. (Bottom) A section of an optical coherence tomography image from along white arrow shown in the Third row middle showing only reduced protrusion of the retinal pigment epithelium. Visual acuity at 12 months was 0.9.

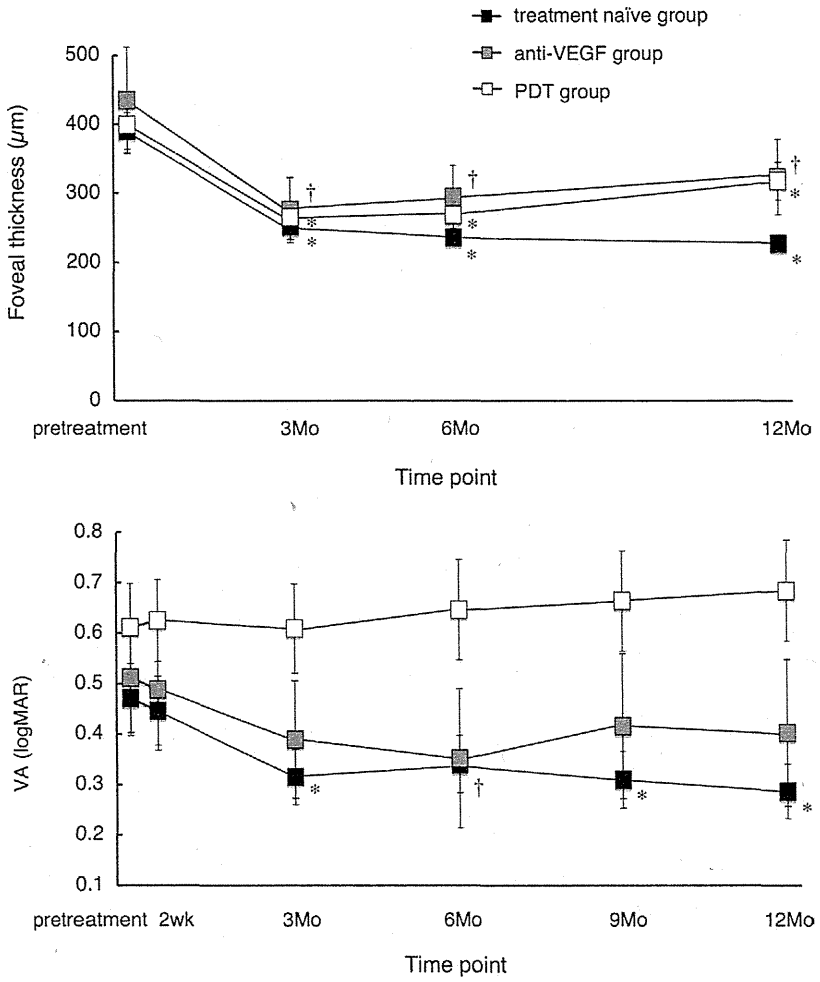

FIGURE 3. Graphs showing (Top) mean foveal thickness and (Bottom) mean visual acuity (in logMAR units) in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT) combined with intravitreal injections of ranibizumab. *P < .01, †P < .05, compared with pretreatment values. logMAR = logarithm of the minimal angle of resolution; VEGF = vascular endothelial growth factor.

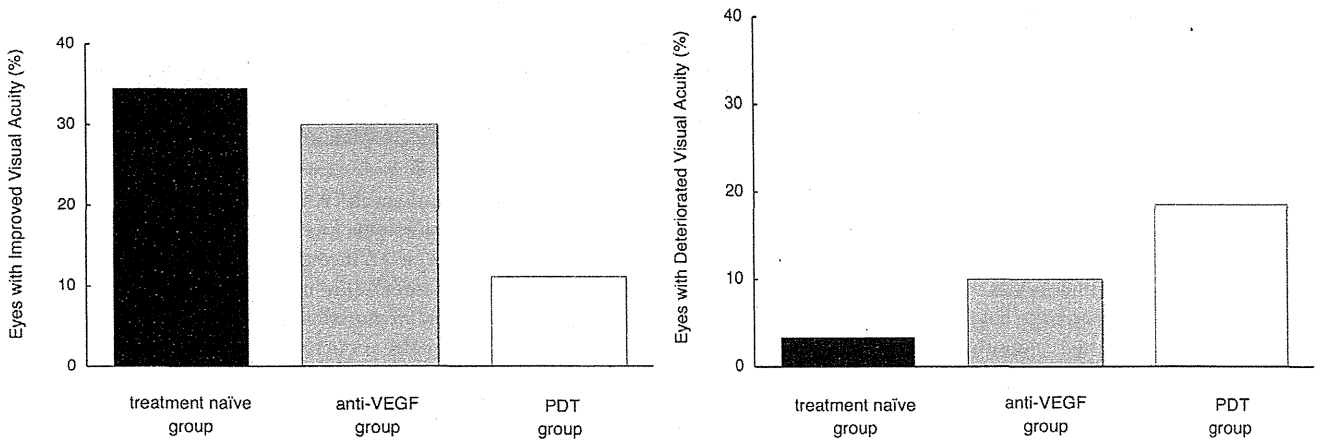

FIGURE 4. Bar graphs showing improvement and deterioration of visual acuity at 12 months after photodynamic therapy (PDT) combined with an intravitreal injection of ranibizumab for the treatment of polypoidal choroidal vasculopathy. Visual acuity was considered to be improved or deteriorated when the change in logMAR units was more than 0.3. logMAR = logarithm of the minimal angle of resolution; VEGF = vascular endothelial growth factor.

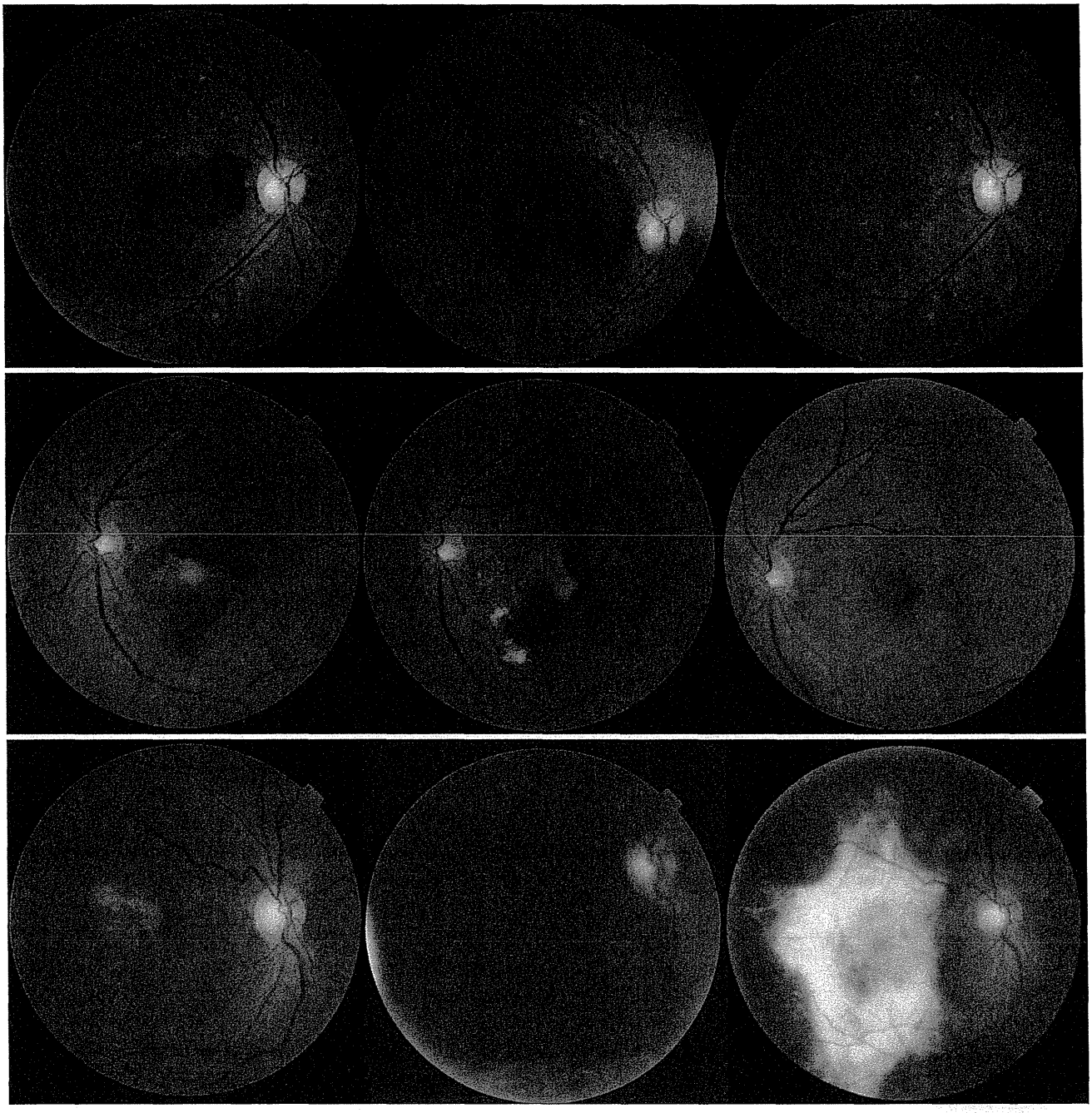

FIGURE 5. Extensive subretinal hemorrhage seen in polypoidal choroidal vasculopathy after photodynamic therapy combined with intravitreal injections of ranibizumab. (Top left) Treatment-naïve polypoidal choroidal vasculopathy before treatment (right eye visual acuity [VA], 0.5). (Top middle) One month after combined therapy, the subretinal hemorrhage has increased (right eye VA, 0.6). (Top right) Subretinal hemorrhage has been absorbed with no treatment. Right eye VA at 12 months was 0.7. (Middle left) Treatment-naïve polypoidal choroidal vasculopathy before treatment (right eye VA, 0.3). (Center) One month after the combined therapy, the subretinal hemorrhage has increased (right eye VA, 0.3). (Middle right) Subretinal hemorrhage has been absorbed with no treatment. Left eye VA at 12 months was 0.5. (Bottom left) Polypoidal choroidal vasculopathy recurred 3 years after the initial photodynamic therapy (right eye VA, 0.3). (Bottom middle) Six weeks after combined therapy, extensive subretinal hemorrhage is seen (right eye VA, 0.01); a vitreous hemorrhage subsequently developed in this eye. (Bottom right) After pars plana vitrectomy, a large disciform scar is seen (left eye VA, 0.02 at 12 months). VA values are expressed in logMAR units.

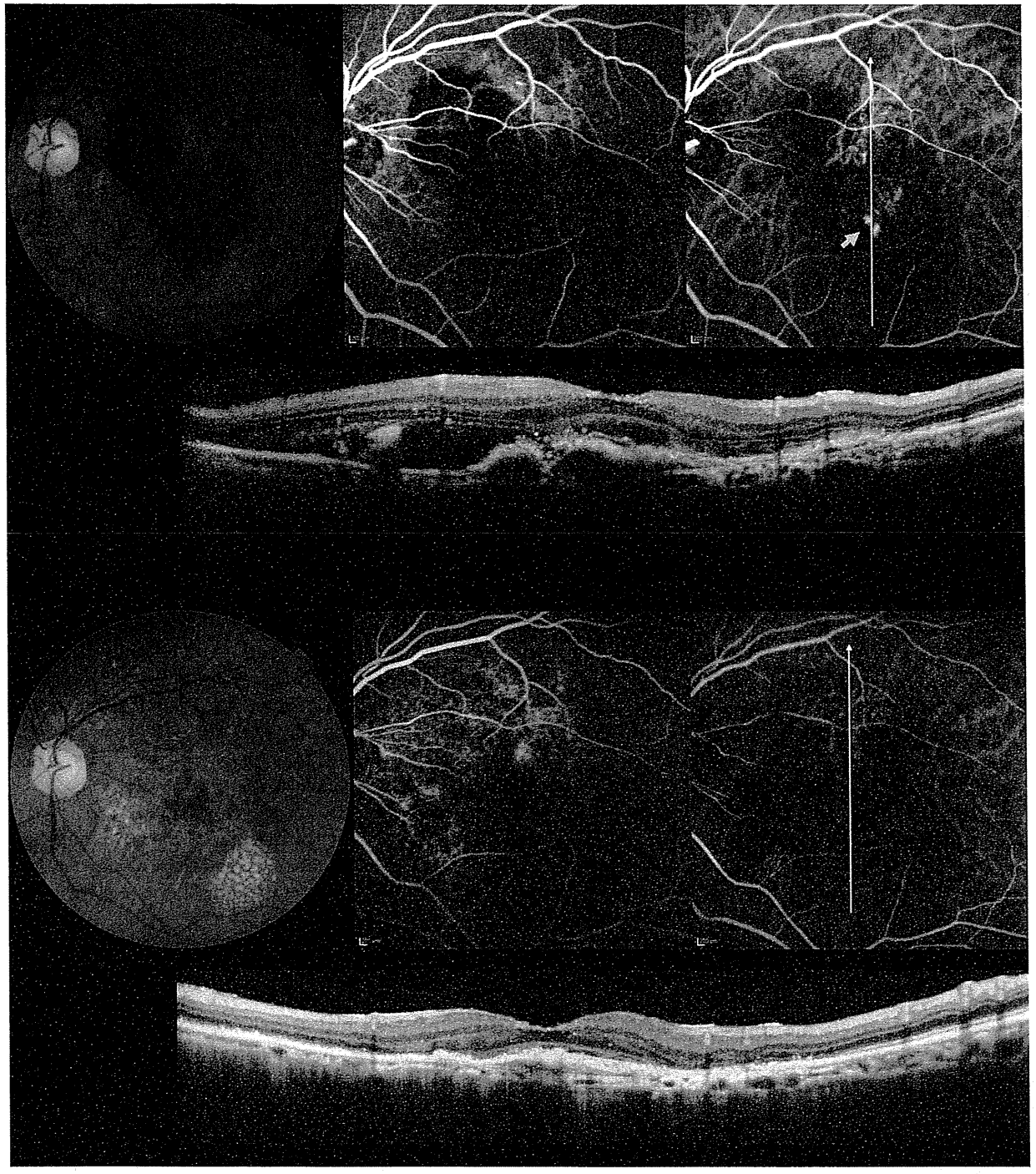

FIGURE 6. Polypoidal choroidal vasculopathy recurred after 2 sessions of photodynamic therapy (PDT). (Top left) Fundus photograph showing multiple serosanguineous pigment epithelial detachments with surrounding subretinal hemorrhage. Visual acuity of the left eye was 0.05. (Top middle) Fluorescein angiography (FA) image revealing occult choroidal neovascularization. (Top right) Indocyanine green angiography (ICGA) image showing a branching vascular network that terminates in polypoidal lesions (yellow arrow). (Second row) Section of an optical coherence tomography image from along the white arrow shown in the Top right revealing protrusions of the retinal pigment epithelium as a result of polypoidal lesions. The left eye was treated with photodynamic therapy combined with an intravitreal injection of ranibizumab. (Third row, left) Three months after treatment, subretinal hemorrhage and exudates have been absorbed. Visual acuity of the left eye was 0.1. (Third row, middle) FA image showing no choroidal neovascularization. (Third row, right) ICGA image showing no polypoidal lesions. (Bottom) Section of an optical coherence tomography scan along the white arrow shown in the Third row right showing minimal protrusion of the retinal pigment epithelium. Visual acuity at 12 months remained at 0.1 in the left eye. VA values are expressed in logMAR units.

epithelial detachment was not included in the lesion area when the absence of underlying CNV was confirmed.

After the initial treatment, each patient was scheduled for an examination at 3 months, at which time they again underwent a comprehensive ophthalmologic examination. When indocyanine green angiography showed recurrent or residual polypoidal lesions and when an exudative change was seen on OCT, additional combination therapy was given. When residual polypoidal lesions were detected on indocyanine green angiography, but no exudative change was seen on OCT, no additional treatment was given and the patient was re-evaluated at the next visit. When only recurrent or residual exudative changes resulting from PCV were seen by OCT examination, but neither polypoidal lesions nor type 2 CNV were seen on angiography, an additional injection of ranibizumab was administered. After the additional injection of ranibizumab, the patient was scheduled to visit our clinic after 1 month, at which time they again underwent a comprehensive ophthalmologic examination.

In the current study, we examined 66 eyes with subfoveal PCV that were treated with PDT combined with intravitreal injections of ranibizumab. Of these 66 eyes, 29 had no history of treatment for PCV (treatment-naïve group). Ten eyes had been treated previously with only intravitreal injections of anti-VEGF agents, including ranibizumab, pegaptanib (Macugen; Pfizer, New York, New York, USA), or bevacizumab (Avastin; Genentech, South San Francisco, California, USA); these eyes had no history of PDT treatment (anti-VEGF group). Twenty-seven eyes had been treated previously with PDT for PCV (PDT group). Of the 27 eyes in the PDT group, 18 also had been treated previously with intravitreal injections of anti-VEGF agents.

All values are presented as mean ± standard deviation. For statistical analysis, best-corrected VA as measured with a Landolt chart was converted to a logarithm of the minimal angle of resolution (logMAR). VA was considered to be improved or deteriorated when the logMAR change was more than 0.3. On OCT sections, foveal thickness was defined as the distance between the inner surface of the neurosensory retina and the retinal pigment epithelium beneath the fovea. In each group, VA or foveal thickness after the combined therapy was analyzed by 1-way repeated-measures analysis of variance with the Dunnett method. At each time point, the change in VA or in foveal thickness was analyzed using the least-significant difference procedure. Software (StatMate III; ATMS, Tokyo, Japan) was used for the statistical analyses. A P value $< .05$ was considered to be statistically significant.

RESULTS

IN THE STUDY DESCRIBED HEREIN, 66 EYES OF 63 PATIENTS (44 men and 19 women), ranging in age from 57 to 85 years (mean ± standard deviation, 72.1 ± 8.0 years), underwent PDT combined with intravitreal injections of ranibizumab for the treatment of PCV. All patients were Japanese, and all were of Asian ancestry. Of these 66 eyes, 29 had no history of treatment for PCV (treatment-naïve group). Ten eyes previously had been treated only with intravitreal injections of anti-VEGF agents (anti-VEGF group); the mean number of previous anti-VEGF treatment was 4.30 ± 2.83 (range, 1 to 9). Twenty-seven eyes had been treated previously for the PCV with PDT (PDT group); the mean number of previous PDT and anti-VEGF treatments was 1.63 ± 0.74 (range, 1 to 3) and 1.67 ± 1.03 (range, 0 to 4), respectively. In the study period over 12 months, the mean number of combined treatments was 1.38 ± 0.63 (range, 1 to 4) (Table 1).

In the treatment-naïve group, all eyes showed an exudative change resulting from the PCV; cystoid macula edema was seen in 9 eyes (31.0%), serous retinal detachment was seen in 27 (93.1%), and serosanguineous pigment epithelial detachment was seen in 12 (41.4%). Initial combined therapy substantially reduced the exudative change. At 3 months, reduced size of the cystoid space and a serous retinal detachment was seen in only 5 eyes and 6 eyes, respectively. Of the 12 eyes with a serosanguineous pigment epithelial detachment, 10 (83.3%) showed a reduction of its size and 5 (41.7%) showed complete resolution at 3 months. Of 24 eyes examined at 3 months with indocyanine green angiography, the polypoidal lesion was reduced in all eyes and had disappeared completely in 19 eyes (79.1%; Figures 1 and 2). However, a branching vascular network remained in all eyes.

Figure 3 shows the change in VA and in foveal thickness after treatment of the treatment-naïve group. Combined therapy reduced substantially the exudative change immediately after initiation of treatment. The foveal thickness was reduced significantly from 390.3 ± 143.2 μm before treatment to 252.9 ± 104.3 μm ($P < .01$) at 3 months and to 227.4 ± 50.3 μm ($P < .01$) at 12 months. The mean VA before treatment (0.47 ± 0.37) also improved significantly, being 0.32 ± 0.30 ($P < .01$) at 3 months. The improvement of VA was maintained until 12 months (0.29 ± 0.29; $P < .01$). At 12 months, significant improvement of VA was seen in 10 eyes (34.5%) and deterioration of VA was seen in 1 eye (3.5%) (Figure 4). Over this 12-month period, additional combined therapy to residual polypoidal lesions with an exudative change was performed in 9 eyes (31.0%). If only an exudative change remained, additional ranibizumab injections were given (7 eyes; 24.1%). In the treatment-naïve group, although 2 eyes showed subretinal hemorrhage (larger than 4 disc areas) after the combined therapy, the hemorrhage was absorbed spontaneously with no decrease in VA (Figure 5). During this 12-month period, no other serious adverse event was seen in the treatment-naïve group.

In the anti-VEGF group, combined therapy reduced substantially the exudative change. The mean foveal thickness was reduced significantly from 434.8 ± 243.2 μm

TABLE 2. Changes in Visual Acuity and Foveal Thickness after Photodynamic Therapy Combined with Intravitreal Injections of Ranibizumab for Polypoidal Choroidal Vasculopathy

	2 Weeks	3 Months	6 Months	9 Months	12 Months
Change in visual acuity (logMAR)					
Total group	−0.01 ± 0.14	−0.09 ± 0.27	−0.07 ± 0.30	−0.06 ± 0.35	−0.07 ± 0.35
Treatment-naïve group	−0.02 ± 0.14	−0.16 ± 0.27	−0.13 ± 0.27	−0.16 ± 0.35	−0.18 ± 0.34
Anti-VEGF group	−0.02 ± 0.14	−0.12 ± 0.20	−0.16 ± 0.26	0.10 ± 0.34	−0.11 ± 0.34
PDT group	0.01 ± 0.14	0.00 ± 0.26[a]	0.03 ± 0.33[a]	0.05 ± 0.33[a]	0.07 ± 0.33[a]
Change in foveal thickness (μm)					—
Total group	—	−139.0 ± 148.3	−141.7 ± 162.1	—	−125.9 ± 189.5
Treatment-naïve group	—	−137.4 ± 150.4	−154.1 ± 141.2	—	−171.3 ± 150.2
Anti-VEGF group	—	−158.7 ± 154.2	−139.3 ± 154.5	—	−123.4 ± 262.4
PDT group	—	−133.4 ± 149.2	−130.1 ± 187.4	—	−81.4 ± 190.8

logMAR = logarithm of the minimal angle of resolution; PDT = photodynamic therapy; VEGF = vascular endothelial growth factor.

The treatment-naïve group consisted of eyes with no previous treatment for polypoidal choroidal vasculopathy (PCV). The anti-VEGF group consisted of eyes that were treated previously with only intravitreal injections of anti-VEGF agents for PCV with no history of PDT treatment. The PDT group consisted of eyes treated previously with PDT for PCV.

[a]$P < .05$, compared with the treatment-naïve group.

TABLE 3. Previous Reports of Photodynamic Therapy and of Combined Therapy of Treatment-Naïve Polypoidal Choroidal Vasculopathy

	Treatment	No. of Eyes	Initial VA	Change in VA at 3 Months	Change in VA at 12 Months	Improvement of VA at 12 Months	Deterioration of VA at 12 Months	No. of PDT Treatments
Treatment-naïve eyes in the current study	PDT+ ranibizumab	29	0.47 ± 0.37	−0.16 ± 0.27	−0.18 ± 0.34	34.5%	3.4%	1.31± 0.47
Otani and associates[28]	PDT	45	0.58 ± 0.37	−0.05 ± 0.17	−0.11 ± 0.30	31.1%	13.3%	1.22 ± 0.47
Gomi and associates[36]	PDT+ bevacizumab	61	0.48	—	−0.12	23.0%	9.8%	1.43
Sato and associates[35]	PDT+ bevacizumab	29	0.43	−0.21 ± 0.27	−0.27 ± 0.34	51.7%	3.4%	1.59
Moon and associates[38]	PDT+ bevacizumab or ranibizumab	22	0.43	−0.07	−0.15	36.4%	9.1%	1.32
Ruamviboonsuk and associates[39]	PDT+ ranibizumab	12	0.725	10.8 letters (ETDRS)	12.3 letters (ETDRS)	58.3%	8.3%	1.9
Rouvas and associates[23]	PDT+ ranibizumab	9	0.81		−0.18	0%	—	1.67

ETDRS = Early Treatment Diabetic Retinopathy Study; PDT = photodynamic therapy; VA = visual acuity.

Visual acuity is presented as logarithm of the minimal angle of resolution (logMAR), if not otherwise indicated. Visual acuity was considered to be improved or deteriorated when the change (logMAR) was more than 0.3.

before treatment to 276.1 ± 148.9 μm ($P < .05$) at 3 months and to 311.4 ± 166.7 μm ($P < .05$) at 12 months. The mean VA was improved from 0.51 ± 0.36 before treatment to 0.39 ± 0.37 at 3 months and to 0.40 ± 0.46 at 12 months, although this improvement was not statistically significant. At 12 months, significant improvement of VA was seen in 3 eyes (30.0%), whereas significant deterioration was seen in 1 eye (10.0%). During the first 12 months, additional combined therapy was administered in 4 eyes (40.0%) and additional ranibizumab injections were given in 1 eye (10.0%). During 12 months, no serious adverse event was seen in any eye of the anti-VEGF group.

In the PDT group, combined therapy reduced substantially the exudative change. The mean foveal thickness was reduced significantly from 399.3 ± 211.8 μm before treatment to 266.0 ± 95.2 μm ($P < .01$) at 3 months and to 318.0 ± 140.6 μm ($P < .01$) at 12 months. With treatment, however, the mean VA did not improve throughout the follow-up period. The mean VA (0.61 ± 0.45 before treatment) remained 0.61 ± 0.46 at 3 months and deteriorated somewhat to 0.68 ± 0.52 at 12 months (Figure 6). At 12 months, significant improvement of VA was seen in only 3 eyes (11.1%), whereas significant deterioration was seen in 5 eyes (18.5%). During 12

months, additional combined therapy was performed in 8 eyes (29.6%) and additional ranibizumab injections were given to in 2 eyes (7.4%). In this PDT group, 3 eyes showed extensive subretinal hemorrhage after treatment. Of these, vitreous hemorrhage developed in 2, necessitating pars plana vitrectomy.

Table 2 shows the change in VA and foveal thickness from pretreatment values in each group. No difference was seen in the change of foveal thickness among the 3 groups. In addition, no significant difference was seen in the change of VA between the treatment-naïve group and the anti-VEGF group. However, change of VA in the PDT group was significantly worse than that of the treatment-naïve group at 3 months and thereafter ($P < .05$).

DISCUSSION

SEVERAL INVESTIGATORS HAVE REPORTED THE EFFECTS of anti-VEGF agents for the treatment of exudative PCV.[13–18,21–24] Cheng and associates recently reported 1-year results of intravitreal injections of bevacizumab for PCV.[24] With a mean of 3.3 injections per affected eye over 12 months, mean VA (logMAR) improved from 0.79 ± 0.42 to 0.67 ± 0.51, although complete resolution of the polypoidal lesions was confirmed in only 16.1% of the eyes. With the use of another anti-VEGF agent, Kokame and associates showed that monthly injections of ranibizumab successfully reduced the exudative changes of PCV.[22] However, even with monthly injections, reduction of the polypoidal lesion was achieved in only 33% of eyes, and the branching vascular network remained in all eyes. Although anti-VEGF agents can reduce the exudative change resulting from PCV, often resulting in VA recovery, their effect on the reduction of the vascular lesions of PCV seems to be limited.[13–24]

In contrast, a number of studies have shown encouraging results of PDT for the vascular lesions of PCV,[26–30] and complete regression of the polypoidal lesions usually is achieved with a small number of PDT sessions.[26–30] In a report by Chan and associates, PDT resulted in complete regression of polypoidal lesion in 95% of eyes with PCV.[27] Furthermore, visual recovery after PDT is reported to be more favorable in eyes with PCV than in those with AMD; Gomi and associates have shown that the median change in VA from baseline to 1 year was −7.0 letters in AMD and 8.0 letters in PCV.[29] However, even if all of the polypoidal lesions regress with PDT, its effect on the branching vascular network remains limited,[28,29] and the remaining branching vascular network may be involved in the recurrence of polypoidal lesions 1 years or even more after successful treatment with PDT.[31–33,41]

In the combination therapy, anti-VEGF agents, which rapidly reduce the exudative change,[13–18,21–24] are expected to contribute to visual recovery in cooperation with regression of the polypoidal lesions induced by PDT.[26–30] In addition, because increased expression of VEGF shortly after PDT has been reported,[42] it seems logical to inject an anti-VEGF agent before PDT treatment.[23,34–36,39] Recently, several investigators reported promising 1-year results of PDT combined with bevacizumab for the treatment of PCV (Table 3).[23,35,36,38] Using PDT in combination with bevacizumab for PCV, Sato and associates reported that the mean improvement in VA was 2.69 lines and that VA improved by 3 lines or more in 51.7% of eyes. Furthermore, using a similar combination of ranibizumab and PDT for 12 eyes with PCV, Ruamviboonsuk and associates achieved a gain in VA of 15 letters or more in 58.3% of eyes at 12 months.[35,39]

Using PDT combined with ranibizumab in treatment-naïve PCV eyes of our patients, the polypoidal lesions disappeared completely in 79.1% of eyes, with a substantial reduction in exudative change. The mean change in VA was −0.16 ± 0.27 at 3 months and was −0.18 ± 0.34 at 12 months. Otani and associates previously reported 1-year results of PDT alone on subfoveal PCV, with inclusion criteria quite similar to those of the current study (Table 3).[28] In that report, the mean number of treatments was no different from that of the present study.[28] However, changes in VA at 3 months (−0.05 ± 0.17) and at 12 months (−0.11 ± 0.30) were less than those in the current study, although the difference was not statistically significant. Our findings are consistent with those recently reported by Gomi and associates, who found that PDT combined with bevacizumab for the treatment of PCV yielded better visual recovery at 1 year than did PDT alone.[36] Thus, for the treatment of PCV, PDT in combination with anti-VEGF agents seems to have a good effect on short-term visual recovery.[34–36,38,39]

Recently, a randomized prospective study (the EVEREST study) has shown the 6-month efficacy of PDT in combination with ranibizumab for PCV (Lai TY, et al. IOVS 2010;51:ARVO E-Abstract 2228). In that study, complete regression of polypoidal lesions was achieved at 6 months in 77.8% after combination therapy, in 71.4% after PDT alone, and in 28.6% after intravitreal injections of ranibizumab. The mean change in VA at 6 months was +10.9, +7.5, and +9.2 for the combination therapy, PDT, and ranibizumab groups, respectively; PCV eyes treated with PDT combined with ranibizumab achieved the highest gains of VA at 6 months. Our results in the treatment-naïve group are quite similar to those of the combination group in the EVEREST study. However, because the current study is retrospective, it is difficult to compare strictly our results with those of the EVEREST study.

In the study described herein, PCV that recurred after previous PDT did, indeed, show a reduction of exudative change, but this did not result in further visual recovery, an observation similarly reported by Romano and associates, who observed that in eyes with PCV that were refractory to treatment by PDT alone, PDT combined with bevaci-

zumab resulted in morphologic stabilization of the PCV lesions, but not in visual recovery.[34] The reason for the poor change in VA seen in the PDT group after the combined therapy is unclear, although damage of the choriocapillaris or retinal pigment epithelium resulting from previous PDT may be involved. Rather, recent reports have shown the association of some genetic factors to the response to PDT and to subsequent visual prognosis in eyes with PCV.[43,44] Originally, the PDT group may consist of the eyes that have genetically poor VA prognosis.

To date, limited information is available on combined therapy for PCV that is refractory to anti-VEGF therapy. The current study has shown that some visual recovery can be expected when PDT is combined with ranibizumab, even in eyes with PCV that previously were treated with anti-VEGF agents. Because the effect of anti-VEGF therapy on polypoidal lesions is limited,[13–18,21–24] combined therapy may well be a treatment option when recurrent or persistent exudative change is seen after anti-VEGF treatments.

For treatment of PCV, one of the most vision-threatening complications of PDT is extensive postoperative hemorrhage.[45,46] In a previous article on PCV treated with PDT, Hirami and associates reported that postoperative subretinal hemorrhage was seen in 28 (30.8%) of 91 eyes, and that bleeding resulted in a vitreous hemorrhage in 6 eyes.[45] Recent reports by Gomi and associates and by Sato and associates suggested a lower incidence of subretinal hemorrhage when the PDT was combined with bevacizumab.[35,36] In the current study, 2 eyes in the treatment-naïve group had an extensive subretinal hemorrhage (larger than 4 disc areas) after such combined therapy, although the hemorrhage was absorbed spontaneously with no decrease in VA. In addition, 3 eyes in the PDT group had an extensive subretinal hemorrhage after treatment, and this resulted in vitreous hemorrhage in 2 eyes, so it is uncertain whether combined therapy actually reduces the posttreatment hemorrhagic complications. However, similar to PDT alone, even if PDT is combined with anti-VEGF agents, physicians need to keep in mind the risk to their patients of these complications.

Major limitations of the current study are its retrospective nature, its small sample size, and its lack of control individuals. Furthermore, we used a Landolt chart, which is based on an uneven spatial gradient scale, for the measurement of VA. However, despite these shortcomings, our findings suggest that PDT combined with intravitreal injections of ranibizumab results in rapid regression of the polypoidal lesions and exudative changes and often results in improvement of VA in eyes with treatment-naïve PCV. Patients with PCV treated with PDT combined with ranibizumab do have a risk of posttreatment hemorrhagic complications. However, because our findings are based on an observation period of only 12 months, it remains unclear whether PDT in combination with ranibizumab reduces the recurrence rate of PCV. Because the long-term efficacy of PDT alone is not as promising as was thought initially, prospective long-term studies are needed to determine the efficacy and safety of combined therapy for PCV.

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