FIGURE 3. Images demonstrating progression of the vascular lesion in larger polypoidal choroidal vasculopathy (PCV). (Top left) Fundus photograph at the initial visit showing a reddish orange nodule with a minute pigment epithelial detachment (PED). (Top right) Sectional image obtained with optical coherence tomography (OCT) along with an arrow seen in the fundus photograph
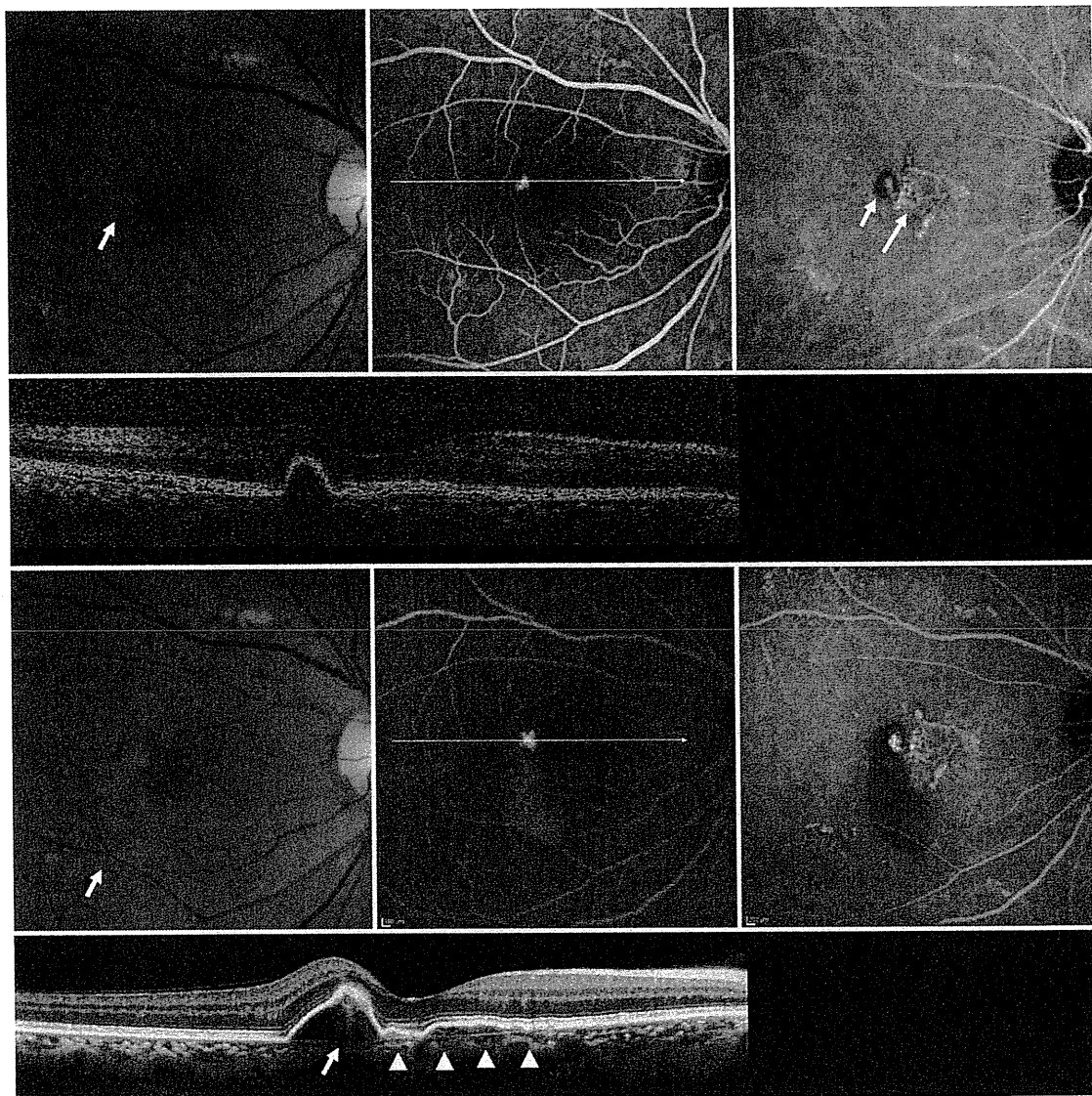
FIGURE 4. Images showing no progression of the vascular lesion in smaller polypoidal choroidal vasculopathy (PCV). (Top left) Fundus photograph at the initial visit showing a small reddish orange nodule (arrow); vision was 20/16. (Top middle) Fluorescein angiogram (FA) obtained at the initial visit showing only a hyperfluorescent spot corresponding to the polypoidal lesion. (Top right) Indocyanine green angiogram showing the vascular components of PCV, which consist of a typical polypoidal lesion (arrow) and a branching vascular network (long arrow). The baseline area of the PCV lesion was 1.77 mm^2. (Second row) Sectional image obtained with optical coherence tomography (OCT) along with the arrow seen in the FA showing a steep protrusion of the retinal pigment epithelium. (Third row left) No treatment was performed. Fundus photograph obtained at 39 months after the initial visit showing a reddish orange nodule with a newly developed serous pigment epithelial detachment; vision was still 20/13. (Third row middle) FA showing a hyperfluorescent spot corresponding to the polypoidal lesion, along with fluorescein pooling in the pigment epithelial detachment. (Third row right) Indocyanine green angiogram revealing no progression of the vascular lesion of PCV. (Bottom) Sectional image obtained with OCT (with the arrow seen on FA) showing protrusion of the retinal pigment epithelium corresponding to the branching network (arrowheads) and steep elevation of the retinal pigment epithelium with moderate inner reflectivity (arrow) corresponding to the polypoidal lesion.

was defined as the distance between the vitreoretinal interface and the tip the outer segment of the inner and outer segments of the photoreceptors.

We genotyped the major AMD- and PCV-associated single nucleotide polymorphism (SNP), rs10490924 (A69S), of ARMS2. Genomic deoxyribonucleic acid was prepared from leukocytes of peripheral blood using a deoxyribonucleic acid extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). The SNPs were genotyped using Taqman SNP assays with the ABI PRISM 7700 system (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions.

TABLE 2. Ocular Manifestations and Complications Seen in Eyes with Polypoidal Choroidal Vasculopathy during Follow-up

	Total (n = 88)	Smaller Polypoidal Choroidal Vasculopathy (n = 22)	Larger Polypoidal Choroidal Vasculopathy (n = 66)	P Value
Suprachoroidal hemorrhage	7	0	7	.1113
Vitreous hemorrhage	8	0	8	.0868
Recurrence	56	11	44	.1620
Type 2 choroidal neovascularization	26	1	25	.0030
Fibrosis	31	4	27	.0533
Serous retinal detachment	81	20	65	.0899
Subretinal hemorrhage (> 1 DA)	55	9	46	.0157
Cystoid macula edema	42	6	36	.0266
Pigment epithelial detachment (> 1 DA)	41	5	36	.0096
Tear of retinal pigment epithelium	8	0	8	.0868

DA = disc area.

One disc area is estimated as 2.54 mm² on the basis of 1 optic disc diameter being 1.8 mm. Based on the area of the lesion at the initial visit, polypoidal choroidal vasculopathy (PCV) eyes were divided into smaller PCV (area of lesion, < 1 DA) and larger PCV (area of lesion, ≥ 1 DA).

Statistical analysis was performed using software designed for this purpose (StatView version 5.0; SAS Institute, Cary, North Carolina, USA). A P value of less than .05 was considered to be statistically significant.

RESULTS

IN THE CURRENT STUDY, 88 EYES OF 88 PATIENTS (60 MEN and 28 women) with PCV, ranging in age from 50 to 86 years (mean ± standard deviation, 70.4 ± 7.5 years), were examined. The follow-up period ranged from 29 to 61 months (mean ± standard deviation, 46.4 ± 8.6 months), and duration from the initial angiogram to the last ranged from 24 to 60 months (mean ± standard deviation, 39.3 ± 9.4 months). All patients were examined with fluorescein and indocyanine green angiography repeatedly during follow-up, ranging from 2 to 11 times (mean ± standard deviation, 4.9 ± 2.0 times). Table 1 shows the characteristics of patients eligible for inclusion in this study. The mean ± standard deviation baseline VA (logarithm of the minimal angle of resolution) was 0.37 ± 0.34. The mean ± standard deviation initial area of the lesion and greatest linear dimension was 7.75 ± 9.78 mm² and 3412 ± 1647 μm, respectively. Figure 1 shows the relationship between area of the lesion, greatest linear dimension, foveal thickness, and VA at initial visit and final examination. Initial area of the lesion (R = 0.801; P < .0001) and initial greatest linear dimension (R = 0.805; P < .0001) showed a close correlation with final measurements.

PCV vascular lesion at the initial visit varied in size, ranging from 0.64 to 63.82 mm². Depending on the initial area of the lesion, we divided the eyes with PCV into 2 groups—the smaller PCV group (baseline area of lesion, <

1 DA; n = 22) and the larger PCV group (baseline area of lesion, ≥ 1 DA; n = 66; Figure 2). The mean area ± standard deviation of the lesion initially was 1.68 ± 0.53 mm² in the smaller PCV group and 9.79 ± 10.55 mm² in the larger PCV group. There were no significant differences in gender, age, or duration of symptoms between groups (P = .5971, P = .3257, and P = .6484, respectively). In addition, there were no differences in the foveal thickness (P = .4628) or thickness of the neurosensory retina in the fovea (P = .4204) at the initial visit. However, the mean initial VA ± standard deviation was significantly better in eyes with smaller PCV (0.24 ± 0.39) than in eyes with larger PCV (0.42 ± 0.35, P = .0383).

During the follow-up period, 64 eyes were treated initially with PDT, and 9 were treated initially with anti-VEGF therapy. Despite these treatments, some eyes with larger PCV showed extension of the vascular component with an exudative change. The mean area of the lesion ± standard deviation in larger PCV progressed significantly from 9.79 ± 10.55 mm² to 16.27 ± 14.19 mm² at the final examination (P < .0001; Figure 3). Furthermore, mean ± standard deviation VA in these eyes deteriorated significantly 0.42 ± 0.35 to 0.76 ± 0.49 at the final examination (P < .0001). In contrast, eyes with smaller PCV lesions often showed minimal progression of the lesion and limited exudative change, and smaller PCV lesions rarely progressed to extensive PCV lesions (Figure 4). However, even in eyes with smaller PCV, the mean lesion size increased during the follow-up period (P = .0037). In smaller PCV, mean ± standard deviation change in the area of the lesion and final area of the lesion were 2.45 ± 3.53 mm² and 4.13 ± 3.59 mm², respectively, which were significantly less than those of the larger PCV (P = .0429 and P = .0002, respectively). In addition, eyes with the smaller PCV showed no decrease in VA (−0.05 ± 0.36;
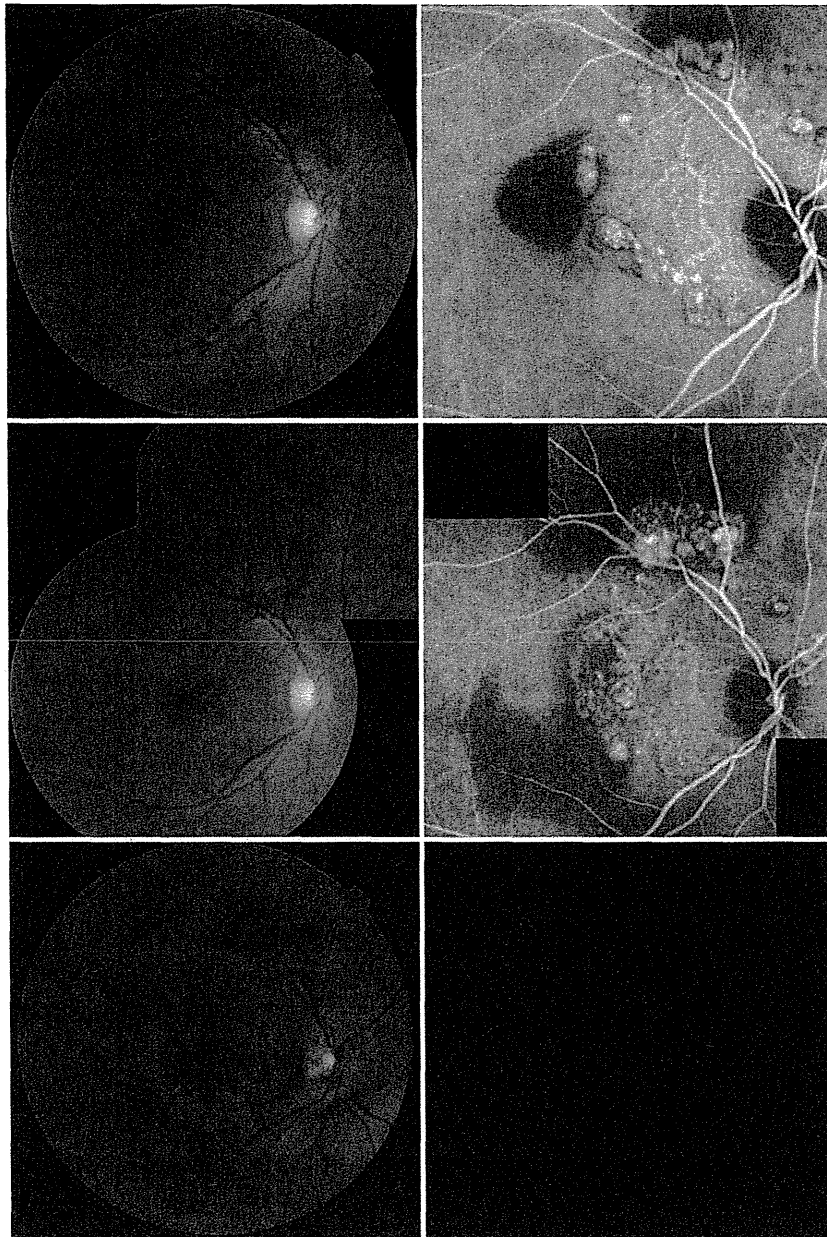
FIGURE 5. Images showing suprachoroidal hemorrhage and vitreous hemorrhage in an eye with larger polypoidal choroidal vasculopathy (PCV). (Top left) Fundus photograph obtained at the initial visit showing multiple large reddish orange nodules; vision was 20/22. (Top right) Indocyanine green angiogram showing a large branching vascular network that terminates with multiple polypoidal lesions. The baseline area of the PCV lesion was 15.80 mm^2. No treatment was performed because this eye had good visual acuity. (Middle left) Fundus photograph obtained at 29 months after the initial visit showing some extension of the vascular components; vision was 20/16. (Middle right) Indocyanine green angiogram showing some extension of the vascular lesion of PCV. (Bottom) Five months after the last angiogram, a sudden suprachoroidal hemorrhage with subsequent vitreous hemorrhage developed in the eye. After vitreous surgery, visual acuity in this eye was only 20/200.

P = .5492) and maintained initial VA to the final visit; mean changes in VA were significantly better in smaller PCV than were those in larger PCV (P = .0015).

Table 2 shows the ocular manifestations and complications seen during follow-up in eyes with smaller or larger PCV. Of the 88 eyes included, 7 (7.6%) showed suprachoroidal hemorrhage and 8 (8.7%) showed vitreous hemor-

rhage (Figure 5), all of which were seen in eyes with larger PCV; no eyes with smaller PCV showed severe complications (P = .1111 and P = .0868). Indeed, in eyes with smaller PCV, even the relatively small amount of subretinal hemorrhage noted (> 1 DA) was seen less frequently than in eyes with larger PCV (P = .0157). In addition, other ocular manifestations associated with severe visual

TABLE 3. ARMS2 Genotypes and Alleles in Patients with Polypoidal Choroidal Vasculopathy

	Smaller Polypoidal Choroidal Vasculopathy (n = 21)	Larger Polypoidal Choroidal Vasculopathy (n = 55)	P Value[a]	Odds Ratio (95% CI)
Genotype			.0397	
TT	3 (10.7%)	25 (89.3%)		5.0 vs GG (3.93 to 6.44)
GT	11 (39.3%)	17 (60.7%)		1.5 vs GG (1.19 to 1.82)
GG	7 (35.0%)	13 (65.0%)		
Allele			.0235	
T	17 (20.2%)	67 (79.8%)		2.4 vs G (2.09 to 2.67)
G	25 (36.8%)	43 (63.2%)		

CI = confidence interval; G = guanine; T = thymine.

One disc area (DA) is estimated as being 2.54 mm^2; this is based on 1 optic disc diameter being 1.8 mm. Based on the area of the lesion at the initial visit, polypoidal choroidal vasculopathy (PCV) eyes were divided into smaller PCV (area of lesion, < 1 DA) and larger PCV (area of lesion ≥ 1 DA).

[a]Chi-square test.

disturbance were seen more often in eyes with larger PCV. Type 2 CNV, subretinal fibrosis, and cystoid macular edema were seen more frequently in eyes with larger PCV (P = .0030, P = .0533, and P = .0266). Of the 88 eyes included in this study, 41 (46.6%) showed a serosanguineous pigment epithelial detachment (area, > 1 DA). Again, a pigment epithelial detachment was seen more frequently in eyes with larger PCV than in those with smaller PCV (P = .0096). Of the 88 eyes of our patients, 8 (8.7%) showed a tear of the retinal pigment epithelium. All of these occurred in eyes with larger PCV; no eyes with smaller PCV showed a tear (P = .0868).

We were able to examine the deoxyribonucleic acid of 76 of the 88 patients. Table 3 shows the distributions of ARMS2 (A69S) genotypes in patients with smaller PCV and in those with larger PCV. There was a significant difference in T allele frequency between patients with smaller PCV and those with larger PCV (20.2% vs 79.8%; P = .0235). In comparison with wild-type homozygosity (GG), homozygosity for the at-risk genotype (TT) increased the likelihood for the larger PCV group by 5.0-fold, and heterozygosity for the at-risk genotype (GT) increased the likelihood for this same group by 1.5-fold.

DISCUSSION

BASED ON THE INITIAL AREA OF THE VASCULAR LESION, WE defined smaller PCV as those with a baseline area of < 1 DA. The remaining PCV, in which the baseline area was ≥ 1 DA, were defined as larger PCV. There were no significant differences in age or duration of symptoms between these 2 groups. The larger PCV, however, did often show progression of the vascular lesions, which in many instances showed an exudative change. In larger

PCV, poor initial VA was even further lessened despite the treatment. In contrast, most eyes diagnosed as having smaller PCV showed only minimal progression of the lesion and limited exudative change. Furthermore, smaller PCV lesions rarely progressed to extensive PCV lesions. Most eyes with smaller PCV had good initial VA and maintained their good VA throughout the follow-up period.

In addition, severe complications such as suprachoroidal hemorrhage, vitreous hemorrhage, and a tear of the retinal pigment epithelium were seen only in eyes with larger PCV; no eyes with smaller PCV showed these severe complications. Furthermore, type 2 CNV and subfoveal fibrosis was seen more frequently in larger PCV. From these findings, we believe that the ocular manifestations, complications, and visual prognosis of smaller PCV are distinct from those of larger PCV. Okubo and associates reported similar findings. Of 13 eyes with PCV that were followed-up for 5 years or longer with no treatment, they reported that eyes with reddish-orange nodules alone or those with nodules plus a small subretinal hemorrhage had a benign clinical course with stable vision.[18]

PCV is thought to have a better visual prognosis than does exudative AMD,[9,20] although the visual prognosis in PCV is not as promising as was thought initially.[21] Previously, direct laser photocoagulation was applied to eyes with PCV.[7,22] Unless the entire vascular lesions could be coagulated, however, the polypoidal lesion often recurred, resulting in decreased VA,[23] although another study showed encouraging short-term results of PDT for PCV.[24] However, 1 year or more after initially successful treatment with PDT, some eyes had a recurrence of PCV and a decrease in VA.[21] Still more recently, although anti-VEGF therapy can reduce the exudative change in PCV shortly after treatment, its effects on the vascular lesions and its

effect on VA seem to be limited.[25–29] In addition, some eyes with large vascular lesions show massive hemorrhagic complications, with sudden visual loss.[30] As shown in Table 2, other vision-threatening complications, such as disciform scar, a tear of the retinal pigment epithelium, or cystoid macular edema, are not as uncommon as reported initially.[16,31,32] Smaller PCV, which often shows a favorable response to treatments and with minimal vision-threatening complications, turn out to have a better visual prognosis than do larger PCV.[17,18]

So far, several classifications of PCV have been reported.[11,12] Based on the location of the lesion, PCV can be categorized as peripapillary, macular, or peripheral.[12] When the vascular lesion is located far from the fovea, visual prognosis tends to be good.[5] Uyama and associates reported 2 patterns of fundus manifestation of PCV, exudative and hemorrhagic.[11] However, they also reported that 36% of the cases had altered their pattern of manifestation during follow-up.[11] Judging from these patterns, it may be difficult to predict visual prognosis.

The polypoidal lesions are thought to be the main source of the exudative change and hemorrhagic complications in PCV.[4,14] Uyama and associates reported 2 patterns of polypoidal lesions: solitary round aneurysmal dilations and a collection of small aneurysmal dilations that resemble a cluster of grapes, the latter of which is associated with a poor prognosis.[11] Clinically, some polypoidal lesions do regress spontaneously, and regress even more after PDT, but these polypoidal lesions recur at the same location or at other terminals of the branching vascular network.[9,12,33] Recently, Cackett and associates reported a classification system of PCV based on polypoidal lesion seen by indocyanine green angiography.[34] In their report,[34] PCV was classified according to size, location, formation, and number of polypoidal lesions. Although objective evaluations are essential for any multicenter study, the branching vascular network, which is one origin of the polypoidal lesions, is essential also to understand status of the disease in each patient.[35]

Based on the results of indocyanine green angiography, Yuzawa and associates reported that there may be 2 types of branching vascular network, one representing an intrachoroidal vascular abnormality, and the other representing neovascularization that grows rapidly in the subretinal pigment epithelial space.[36] Unfortunately, histologic reports of PCV are limited,[37,38] although recent OCT findings support the theory that the vascular components of PCV are located within or above the Bruch membrane. In Figure 4, both the polypoidal lesion and the branching vascular network are seen between the retinal pigment epithelium and Bruch membrane,[39] suggesting that the vascular lesions in PCV are a form of CNV.[14]

The branching vascular network, which only rarely disappeared with treatment, tended to extend over time.[35] It is thought that progression of PCV is slower than that of exudative AMD.[9] Yannuzzi and associates reported that the branching vascular network enlarges by simple proliferation and hypertrophy of the vascular components, by conversion of the polypoidal lesion into the advancing edge of a vascular channel, or by the unfolding of a cluster of polypoidal lesions and subsequent transformation into enlarging vascular tubular components.[9] In the current study, based on the initial size of the vascular components, we divided PCV into smaller and larger types, a classification that provides useful information to both physicians and patients regarding the risk of severe complications and visual prognosis. Smaller PCV rarely progress to become extensive lesions and often have a more favorable clinical course that is quite different from that of larger PCV.[17,18] In the current study, all 15 eyes in which extensive vascular lesions (more than 8 DA) finally developed had a lesion of more than 3 DA at the initial examination. It follows that all eyes with extensive PCV already had shown relatively large vascular lesion when they had initial visual symptoms. From the current study, we could not provide any information on the beginning of the extensive PCV.

In the current study, we examined the genotypes of rs10490924 (A69S) of ARMS2 in patients with smaller PCV (21 patients) and those with larger PCV (55 patients). An increasing number of reports showed that ARMS2 A69S is associated strongly with AMD, as well as with typical AMD and with PCV.[40–45] In the current study, there was a significant difference in T allele frequency between the smaller PCV and the larger PCV (20.2% vs 79.8%). Our findings suggest that smaller PCV is different from larger PCV, not only from the clinical point of view but also from the genetic point of view.

In PCV patients, Sakurada and associates reported a difference in the genotypic frequency at this site between eyes with and without vitreous hemorrhage, with the frequency of the T allele being significantly greater in the vitreous hemorrhage group than that in the nonvitreous hemorrhage group.[40] In another report, Sakurada and associates reported that this genotype is not associated with lesion composition or size as seen by indocyanine green angiography.[46] However, they did indicate that the T allele at this SNP is associated with the exudative activity of polypoidal lesions.[46] Although further studies are necessary to elucidate the contribution of this SNP to the progression of the vascular lesion, this SNP seems to be associated with the occurrence of PCV itself and with the activity of the vascular lesions.

Limitations of the current study are its retrospective nature and the various treatment regimens used. Our patents received primarily PDT or anti-VEGF therapy.[25–29] Because recent studies have suggested that PDT in combination with anti-VEGF therapy may be the most promising treatment of PCV, it is possible that our patients did not receive the most effective treatment.[47,48] In the current study, 8 eyes showed a relatively large subretinal hemorrhage at the initial visit. It may be possible that the lesion size was somewhat underestimated in a few eyes.

However, because all of these 8 eyes were classified as having larger PCV, there is no possibility that any eye with a relatively large subretinal hemorrhage was classified incorrectly as having a smaller PCV. In addition, 11 of 22 eyes with smaller PCV still had small lesions (< 1 DA) at the final examination. In remaining 11 eyes, however, the final area of the lesion was more than 1 DA, although the lesion remained relatively small and visual prognosis often was good. If these eyes had been examined later, they might have been classified as having larger PCV. However, despite these shortcomings, our findings suggest that most smaller PCV show limited progression and that good visual function is maintained—with no serious complications. Furthermore, it may be of use to differentiate this type of PCV from larger PCV to prognosticate visual prognosis in affected individuals.

REFERENCES

1. Kleiner RC, Brucker AJ, Johnston RL. The posterior uveal bleeding syndrome. Retina 1990;10(1):9–17.
2. Stern RM, Zakov ZN, Zegarra H, Gutman FA. Multiple recurrent serosanguineous retinal pigment epithelial detachments in black women. Am J Ophthalmol 1985;100(4):560–569.
3. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCV). Retina 1990; 10(1):1–8.
4. Spaide RF, Yannuzzi LA, Slakter JS, Sorenson J, Orlach DA. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. Retina 1995;15(2):100–110.
5. Yannuzzi LA, Nogueira FB, Spaide RF, et al. Idiopathic polypoidal choroidal vasculopathy: a peripheral lesion. Arch Ophthalmol 1998;116(3):382–383.
6. Moorthy RS, Lyon AT, Rabb MF, Spaide RF, Yannuzzi LA, Jampol LM. Idiopathic polypoidal choroidal vasculopathy of the macula. Ophthalmology 1998;105(8):1380–1385.
7. Uyama M, Matsubara T, Fukushima I, et al. Idiopathic polypoidal choroidal vasculopathy in Japanese patients. Arch Ophthalmol 1999;117(8):1035–1042.
8. Akman A, Akbatur H, Yilmaz G, Aydin P. Peripheral idiopathic polypoidal choroidal vasculopathy. Eye 2000; 14(Pt 4):680–681.
9. Yannuzzi LA, Ciardella A, Spaide RF, Rabb M, Freund KB, Orlock DA. The expanding clinical spectrum of idiopathic polypoidal choroidal vasculopathy. Arch Ophthalmol 1997; 115(4):478–485.
10. Sho K, Takahashi K, Yamada H, et al. Polypoidal choroidal vasculopathy: incidence, demographic features, and clinical characteristics. Arch Ophthalmol 2003;121(10):1392–1396.
11. Uyama M, Wada M, Nagai Y, et al. Polypoidal choroidal vasculopathy: natural history. Am J Ophthalmol 2002; 133(5):639–648.
12. Ciardella AP, Donsoff IM, Huang SJ, Costa DL, Yannuzzi LA. Polypoidal choroidal vasculopathy. Surv Ophthalmol 2004;49(1):25–37.
13. Ahuja RM, Stanga PE, Vingerling JR, Reck AC, Bird AC. Polypoidal choroidal vasculopathy in exudative and haem-

orrhagic pigment epithelial detachments. Br J Ophthalmol 2000;84(5):479–484.
14. Tsujikawa A, Sasahara M, Otani A, et al. Pigment epithelial detachment in polypoidal choroidal vasculopathy. Am J Ophthalmol 2007;143(1):102–111.
15. Gomi F, Sawa M, Mitarai K, Tsujikawa M, Tano Y. Angiographic lesion of polypoidal choroidal vasculopathy on indocyanine green and fluorescein angiography. Graefes Arch Clin Exp Ophthalmol 2007;245(10):1421–1427.
16. Tateiwa H, Kuroiwa S, Gaun S, Arai J, Yoshimura N. Polypoidal choroidal vasculopathy with large vascular network. Graefes Arch Clin Exp Ophthalmol 2002;240(5):354–361.
17. Okubo A, Hirakawa M, Ito M, Sameshima M, Sakamoto T. Clinical features of early and late stage polypoidal choroidal vasculopathy characterized by lesion size and disease duration. Graefes Arch Clin Exp Ophthalmol 2008;246(4):491–499.
18. Okubo A, Arimura N, Abematsu N, Sakamoto T. Predictable signs of benign course of polypoidal choroidal vasculopathy: based upon the long-term observation of nontreated eyes. Acta Ophthalmol 2010;88(4):107–114.
19. Okubo A, Ito M, Kamisasanuki T, Sakamoto T. Visual improvement following trans-Tenon's retrobulbar triamcinolone acetonide infusion for polypoidal choroidal vasculopathy. Graefes Arch Clin Exp Ophthalmol 2005;243(8):837–839.
20. Maruko I, Iida T, Saito M, Nagayama D, Saito K. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. Am J Ophthalmol 2007;144(1):15–22.
21. Kurashige Y, Otani A, Sasahara M, et al. Two-year results of photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol 2008;146(4):513–519.
22. Nishijima K, Takahashi M, Akita J, et al. Laser photocoagulation of indocyanine green angiographically identified feeder vessels to idiopathic polypoidal choroidal vasculopathy. Am J Ophthalmol 2004;137(4):770–773.
23. Yuzawa M, Mori R, Haruyama M. A study of laser photocoagulation for polypoidal choroidal vasculopathy. Jpn J Ophthalmol 2003;47(4):379–384.
24. Chan WM, Lam DS, Lai TY, et al. Photodynamic therapy with verteporfin for symptomatic polypoidal choroidal vas-

culopathy: one-year results of a prospective case series. Ophthalmology 2004;111(8):1576–1584.

25. Lee SY, Kim JG, Joe SG, Chung H, Yoon YH. The therapeutic effects of bevacizumab in patients with polypoidal choroidal vasculopathy. Korean J Ophthalmol 2008; 22(2):92–99.

26. Gomi F, Sawa M, Sakaguchi H, et al. Efficacy of intravitreal bevacizumab for polypoidal choroidal vasculopathy. Br J Ophthalmol 2008;92(1):70–73.

27. Lai TY, Chan WM, Liu DT, Luk FO, Lam DS. Intravitreal bevacizumab (Avastin) with or without photodynamic therapy for the treatment of polypoidal choroidal vasculopathy. Br J Ophthalmol 2008;92(5):661–666.

28. Tsujikawa A, Ooto S, Yamashiro K, Tamura H, Otani A, Yoshimura N. Treatment of polypoidal choroidal vasculopathy by intravitreal injection of bevacizumab. Jpn J Ophthalmol 2010;54(4):310–319.

29. Kokame GT, Yeung L, Lai JC. Continuous anti-VEGF treatment with ranibizumab for polypoidal choroidal vasculopathy: 6-month results. Br J Ophthalmol 2010;94(3):297–301.

30. Hirami Y, Tsujikawa A, Otani A, et al. Hemorrhagic complications after photodynamic therapy for polypoidal choroidal vasculopathy. Retina 2007;27(3):335–341.

31. Tsujikawa A, Hirami Y, Nakanishi H, et al. Retinal pigment epithelial tear in polypoidal choroidal vasculopathy. Retina 2007;27(7):832–838.

32. Tamura H, Tsujikawa A, Otani A, et al. Polypoidal choroidal vasculopathy appearing as classic choroidal neovascularisation on fluorescein angiography. Br J Ophthalmol 2007; 91(9):1152–1159.

33. Otani A, Sasahara M, Yodoi Y, et al. Indocyanine green angiography: guided photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol 2007;144(1): 7–14.

34. Cackett P, Wong D, Yeo I. A classification system for polypoidal choroidal vasculopathy. Retina 2009;29(2):187–191.

35. Lim TH, Laude A, Tan CS. Polypoidal choroidal vasculopathy: an angiographic discussion. Eye 2010;24(3):483–490.

36. Yuzawa M, Mori R, Kawamura A. The origins of polypoidal choroidal vasculopathy. Br J Ophthalmol 2005;89(5):602–607.

37. Lafaut BA. Clinicopathologic correlation of surgically removed submacular tissue. Bull Soc Belge Ophtalmol 2000(278):49–53.

38. Rosa RH Jr, Davis JL, Eifrig CW. Clinicopathologic reports, case reports, and small case series: clinicopathologic correlation of idiopathic polypoidal choroidal vasculopathy. Arch Ophthalmol 2002;120(4):502–508.

39. Ojima Y, Hangai M, Sakamoto A, et al. Improved visualization of polypoidal choroidal vasculopathy lesions using spectral-domain optical coherence tomography. Retina 2009; 29(1):52–59.

40. Sakurada Y, Kubota T, Mabuchi F, Imasawa M, Tanabe N, Iijima H. Association of LOC387715 A69S with vitreous hemorrhage in polypoidal choroidal vasculopathy. Am J Ophthalmol 2008;145(6):1058–1062.

41. Lima LH, Schubert C, Ferrara DC, et al. Three major loci involved in age-related macular degeneration are also associated with polypoidal choroidal vasculopathy. Ophthalmology 2010;117(8):1567–1570.

42. Mori K, Horie-Inoue K, Gehlbach PL, et al. Phenotype and genotype characteristics of age-related macular degeneration in a Japanese population. Ophthalmology 2010;117(5):928–938.

43. Lee KY, Vithana EN, Mathur R, et al. Association analysis of CFH, C2, BF, and HTRA1 gene polymorphisms in Chinese patients with polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2008;49(6):2613–2619.

44. Kondo N, Honda S, Ishibashi K, Tsukahara Y, Negi A. LOC387715/HTRA1 variants in polypoidal choroidal vasculopathy and age-related macular degeneration in a Japanese population. Am J Ophthalmol 2007;144(4):608–612.

45. Gotoh N, Nakanishi H, Hayashi H, et al. ARMS2 (LOC387715) variants in Japanese patients with exudative age-related macular degeneration and polypoidal choroidal vasculopathy. Am J Ophthalmol 2009;147(6):1037–1041.

46. Sakurada Y, Kubota T, Imasawa M, et al. Angiographic lesion size associated with LOC387715 A69S genotype in subfoveal polypoidal choroidal vasculopathy. Retina 2009; 29(10):1522–1526.

47. Ogino T, Takeda M, Imaizumi H, Okushiba U. Photodynamic therapy for age-related macular degeneration in Japanese patients: results after one year. Jpn J Ophthalmol 2007;51(3):210–215.

48. Sato T, Kishi S, Matsumoto H, Mukai R. Combined photodynamic therapy with verteporfin and intravitreal bevacizumab for polypoidal choroidal vasculopathy. Am J Ophthalmol 2010; 149(6):947–954.

Biosketch

Akitaka Tsujikawa, MD, is a graduate of the Kyoto University Graduate School of Medicine, Kyoto, Japan. He completed his ophthalmology residency at Kyoto University Hospital and a fellowship at the Kurashiki Central Hospital in Japan. Following fellowship, he worked on the retinal microcirculation at the Kyoto University Graduate School of Medicine and at the Children's Hospital in Boston. He currently specializes in macular diseases and retinal vascular diseases at the Kyoto University Graduate School of Medicine.

Genetic Variants in Pigment Epithelium-Derived Factor Influence Response of Polypoidal Choroidal Vasculopathy to Photodynamic Therapy

Isao Nakata, MD,[1,2] Kenji Yamashiro, MD, PhD,[1] Ryo Yamada, MD, PhD,[2] Norimoto Gotoh, MD,[1,2]
Hideo Nakanishi, MD, PhD,[1,2] Hisako Hayashi, MD,[1,2] Akitaka Tsujikawa, MD, PhD,[1]
Atsushi Otani, MD, PhD,[1] Sotaro Ooto, MD, PhD,[1] Hiroshi Tamura, MD, PhD,[1] Masaaki Saito, MD, PhD,[3]
Kuniharu Saito, MD,[3] Tomohiro Iida, MD, PhD,[3] Akio Oishi, MD, PhD,[4] Yasuo Kurimoto, MD, PhD,[4]
Fumihiko Matsuda, PhD,[2] Nagahisa Yoshimura, MD, PhD[1]

Purpose: To investigate whether photodynamic therapy (PDT) outcomes of polypoidal choroidal vasculopathy (PCV) are related to baseline clinical characteristics, smoking history, or genetic factors by analyzing the retreatment-free period after the first PDT.

Design: Retrospective cohort study.

Participants: The study consisted of 167 patients with PCV who underwent PDT as their first treatment.

Methods: We targeted 638 single nucleotide polymorphisms (SNPs) in 42 possible susceptible genes for age-related macular degeneration to evaluate their relation to the effectiveness of PDT for PCV. For this evaluation, we used 2 methods: (1) survival analysis, with the retreatment-free period as the target; and (2) logistic regression test between the need for additional therapy within 3 months after the first PDT and the genotypes, with age, gender, smoking status, and greatest linear dimension (GLD) at baseline as covariates. The contributions of smoking status and GLD at baseline for the retreatment-free period also were evaluated. Contributions of these factors to visual prognosis were evaluated for 1 year after PDT.

Main Outcome Measures: Retreatment-free period after the first PDT for PCV. Secondary outcome measures included correlation of the susceptible factor to the retreatment requirement within the 3-month follow-up and the mean visual acuity change.

Results: In survival analyses, SERPINF1 rs12603825 showed a significant association with the retreatment-free period after the first PDT; those patients homozygous for the minor allele A of rs12603825 received additional treatment after PDT within significantly shorter times than those with other genotypes ($P = 0.0038$). There was no significant difference in the retreatment-free period between baseline GLD and smoking status. Retreatment within 3 months was required significantly more in patients with the AA genotype, even after taking into consideration the effect of clinical characteristics (age, gender), baseline PCV lesion size, and smoking status ($P = 0.0027$). Furthermore, patients with the AA genotype showed significantly worse visual prognosis after PDT ($P = 0.013$).

Conclusions: Pigment epithelium-derived factor (SERPINF1 or PEDF) polymorphisms may influence the initial response to and visual prognosis after PDT for PCV. Our findings may lead to understanding the pathogenesis of PCV and modification of the effects of PDT.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. *Ophthalmology 2011;118:1408–1415 © 2011 by the American Academy of Ophthalmology.*

Polypoidal choroidal vasculopathy (PCV) is observed frequently in Asian patients diagnosed with exudative age-related macular degeneration (AMD),[1,2] and PCV recently has been considered to be a separate clinical entity differing from neovascular AMD and other diseases associated with subretinal neovascularization.[3] Recent studies on the genetics of AMD and PCV have recognized them as complex diseases caused by the actions and interactions of numerous genes and environmental factors.[4–8]

Photodynamic therapy (PDT) with verteporfin was previously one of the main therapeutic options for neovascular AMD, and several studies have shown that the treatment effects of PDT for AMD vary according to the baseline composition, including lesion size of choroidal neovascularization, visual acuity, and genotype.[9–12] Many studies have reported that PDT is more effective in treating PCV than neovascular AMD,[13–15] although PDT for PCV often has to be repeated, either because of persistent disease or

recurrence.[16,17] There are limited reports of the association between clinical or pathologic features and the response of PCV to PDT. When evaluating the effect of PDT for PCV, it is essential to consider both genetic and environmental factors, which has been done in the evaluation of AMD. As shown in the AMD study, studies have shown that smoking is associated with the development of PCV.[18–20]

The objectives of the current study were to discern whether the response of PCV to PDT was related to baseline clinical characteristics, smoking history, and genetic background by analyzing multiple single nucleotide polymorphisms (SNPs) and focusing primarily on the clinical retreatment-free period.

Materials and Methods

All procedures in this study adhered to the tenets of the Declaration of Helsinki. The institutional review board and ethics committee of each institute involved approved the protocols of this study. All patients were fully informed of the purpose and procedures of this study, and written consent was obtained from each patient.

Patients and Methods

The study consisted of 167 Japanese patients with PCV who underwent PDT at Kyoto University Hospital, Fukushima Medical University Hospital, or Kobe City Medical Center General Hospital between August 2004 and February 2009. All patients enrolled in the study met the criteria of PCV as proposed by the Japanese Study Group of Polypoidal Choroidal Vasculopathy.[21] Each subject underwent a complete ophthalmic examination, including measurement of best-corrected visual acuity, indirect ophthalmoscopy and slit-lamp biomicroscopy with a contact lens by a retina specialist, fluorescein angiography and indocyanine green angiography (ICGA), and optical coherence tomography. Best-corrected visual acuity was measured with a Landolt chart and converted to a logarithm of the minimal angle of resolution for statistical analysis. The inclusion criteria for this study were (1) diagnosis of PCV, (2) treatment with PDT as the first therapy, (3) age ≥ 50 years, (4) presence of a subfoveal lesion, and (5) best-corrected Snellen visual acuity equivalent of 20/200 to 20/40 at baseline. Exclusion criteria were (1) choroidal neovascularization caused by other diseases (e.g., pathologic myopia, uveitis) and (2) combined treatment (e.g., PDT in combination with antivascular endothelial growth factor drugs). If a patient had bilateral PCV treated with PDT, the eye treated earlier that fulfilled the criteria of this study was selected as the study eye for analysis. The greatest linear dimension (GLD) used for PDT was based on the ICGA findings and covered the entire PCV vascular lesion, including polypoidal lesions and branching vascular network vessels.[22] All patients received PDT with verteporfin following the standard protocol of treatment[23] except for determination of the GLD. At 3 months after the first PDT for PCV, all patients underwent a repeat ophthalmologic examination, including optical coherence tomography or fluorescein angiography and ICGA, on which the need for additional treatment was based. This sequence was followed during the follow-up time at intervals of patient visits to the outpatient clinic for up to 3 months. The retreatment-free period was calculated as the date of the first PDT to the date that the treating physician opted for additional treatment for a persistent or new lesion.

To evaluate the effect of GLD size, patients were divided into 3 groups according to the guidelines for PDT in Japan.[24] The GLD was ≤1800 μm in the first group, 1800 to 5400 μm in the second group, and ≥5400 μm in the third group. Information on smoking status (never smoked, ex-smoker, or current smoker) was obtained by self-reported questionnaire.

Two methods were used for the current PDT study: (1) survival analysis, with the retreatment-free period after the first PDT being the target; and (2) logistic regression test between 2 subgroups to evaluate the initial response to PDT. Because additional treatment with PDT is usually considered at 3 months after the first PDT,[14,25] the patients were classified into 1 of 2 groups by whether additional treatment was required within the first 3-month follow-up. Those patients who required additional therapy within 3 months after the first PDT (i.e., they continued to show an exudative lesion or had a worsened exudative lesion) were regarded as having a retreatment-free period of less than 3 months (Fig 1).

Multiplexing Single Nucleotide Polymorphism Analysis

To identify susceptible SNPs for the retreatment-free period after the first PDT, we used 31 of 160 PCV samples that were genotyped with the Illumina GoldenGate assay across 638 SNPs of 42 genes on a BeadStation 500G Genotyping System (Illumina, Inc., San Diego, CA); this was customized to evaluate possible AMD/PCV susceptible genes (listed in Table 1, available at http://aaojournal. org.). Haploview[26] software was used to infer the linkage disequilibrium (LD) in the targeted regions; among the candidate SNPs, LD indices (D' and r^2) were calculated with Haploview. To detect an association between the gene and the response to PDT, 1 representative SNP was chosen from each region. To confirm the positive association seen in the screening samples, 136 additional patients were genotyped for the SNPs with the Taqman SNP assay, which used the ABI PRISM 7700 system (Applied Biosystems, Foster City, CA). The 31 PCV samples used in the initial screening were also genotyped to validate concordance between the GoldenGate assay and the Taqman assay. Samples with a low successful call rate (<95%) were excluded from the study.

Statistical Analyses

Survival analysis was conducted using Kaplan–Meier methods to estimate differences among genotypes in the retreatment-free period after the first PDT. The retreatment-free period of the patients with no additional treatment was censored at the time of last contact. To detect differences in survival, Breslow–Gehan–Wilcoxon tests were used. When a significant association was found, the best fitting model (additive, dominant, or recessive) was then investigated. The Hardy–Weinberg equilibrium for genotypic distribution was evaluated using the Hardy–Weinberg equilibrium exact test. Descriptive statistics for all demographic and clinical variables were calculated and comparisons were made using the unpaired *t* test for means with continuous data (e.g., age) and the chi-square test for categoric data (e.g., gender). Logistic regression analysis was used to evaluate the association for adjusting age, gender, smoking status, GLD, and genotype considering the best fitting model. Visual prognosis after treatment was compared by a repeated-measures analysis of variance. *P* value correction was performed with the Bonferroni method using the ratio of the number of all genotyped SNPs in the screening procedure. For overall survival analysis, *P* value correction was performed with the Bonferroni method using the ratio of the number of
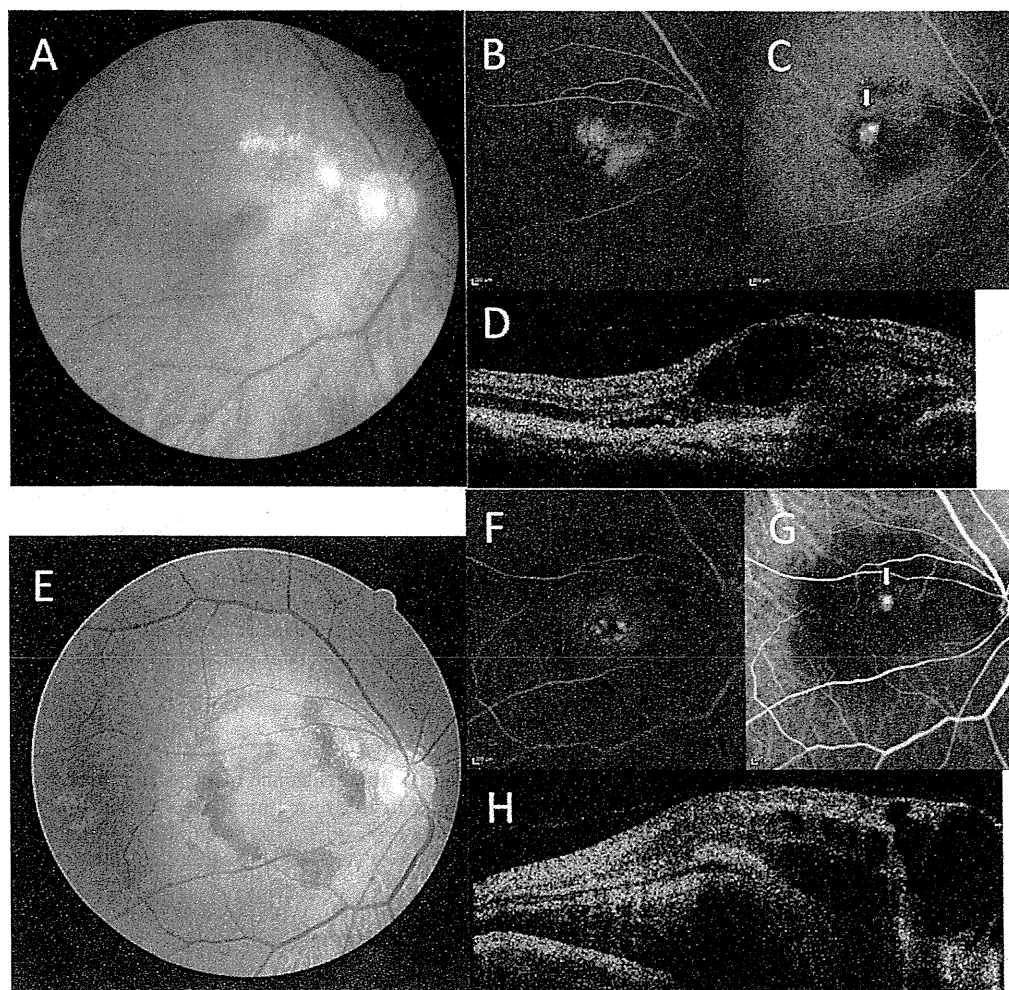
Figure 1. Fundus photographs (A, E), fluorescein angiographs (B, F), indocyanine green angiographs (C, G), and optical coherence tomographs (D, H) of a patient who received additional treatment within 3 months after the first PDT. This 72-year-old man with PCV in his right eye underwent PDT as his first therapy. Before the treatment (A–D), his best-corrected visual acuity was 20/200 and ICGA revealed an active polyp (C, *white arrow*). Seventy-six days after PDT (E–H), the treating physician opted to perform additional treatment because his best-corrected visual acuity decreased to 20/400, a new macular hemorrhage appeared, and the polyp (G, *white arrow*) and exudative lesion (F, H) remained active.

selected SNPs from the screening. Significance was defined at the 5% level.

Results

A total of 167 patients with PCV who underwent PDT as their first therapy at 1 of 3 institutes were enrolled in the current study. Demographic and clinical characteristics of each patient by institute involved are shown in Table 2.

Survival Analysis for the Retreatment-free Period

Of the 160 patients with PCV who were genotyped by the Illumina GoldenGate assay, which launches 638 SNPs across 42 genes in our previous study, 31 met the inclusion criteria of the current PDT study and were used for the screening of genotype data. Because 57 SNPs with no call or scattered or overlapping clusters were excluded from the analysis, 581 SNPs were evaluated by survival analysis with the retreatment-free period. We identified 6 SNPs in

4 genes (FBLN5, CX3CR1, SERPINF1, and TLR4), with the P value adjusted for multiple testing <0.05 (Table 3). At SERPINF1 gene, rs12103559 and rs1894286 were in strong LD (pair-wise D' = 1.0 and r^2 = 1.0). By considering the LD and minor allele frequency of 3 SNPs of this region, we selected rs12603825 as the representative SNP of the SERPINF1 gene and tested a total of 4 SNPs in other patients. A total of 136 additional patients from the 3 institutes were genotyped by the Taqman method. Genotyping success rates of the 4 SNP markers in the additional 136 samples were greater than 98.8%. In overall survival analyses, SERPINF1 rs12603825 showed a significant association with the retreatment-free period (P = 0.0117). Patients homozygous for the minor allele of rs12603825 (i.e., a recessive model) were given an additional treatment after the first PDT in significantly shorter time periods than were the other genotypes (P = 0.0038), and this association remained significant after a permutation procedure for multiple test correction (corrected P = 0.015) (Table 3, Fig 2).

There was no significant difference in the retreatment-free period among the 3 GLD groups and the smoking status groups

Table 2. Baseline Characteristics of the Study Population

	Kyoto	Kobe City	Fukushima	Total
No. of patients	79	51	37	167
Mean age (yrs)	73.01	70.92	70.64	71.86
Gender				
Women	21 (26.6)	18 (35.3)	8 (21.6)	47 (28.1)
Men	58 (73.4)	33 (64.7)	29 (78.4)	120 (71.9)
Mean visual acuity (logMAR)	0.552	0.605	0.573	0.573
Smoking history				
Never	26 (36.1)	22 (44.9)	15 (40.5)	63 (39.9)
Previous	27 (37.5)	21 (42.9)	12 (32.5)	60 (38.0)
Current	19 (26.4)	6 (12.2)	10 (27.0)	35 (22.1)
Mean follow-up (days)	1156.4	1084.6	1198.8	1143.8
GLD				
≤1800 μm	13 (16.9)	3 (6.3)	7 (18.9)	23 (14.2)
1800–5400 μm	60 (77.9)	41 (85.4)	28 (75.7)	129 (79.6)
>5400 μm	4 (5.2)	4 (8.3)	2 (6.4)	10 (6.2)
Mean (μm)	2817.5	3476.7	3150.5	3209.0

GLD = greatest linear dimension; logMAR = logarithm of the minimal angle of resolution.

based on overall survival analysis (P = 0.214 and 0.166, respectively), although borderline evidence of an association was observed between never smoked and ex-smokers plus current smokers (P = 0.060) (Fig 3).

Effect of Photodynamic Therapy

We investigated the association between the susceptible SNP for the retreatment-free period and initial clinical response to PDT. Of the 167 eyes eligible for this analysis, 13 required additional treatment within 3 months after their first PDT, and 150 did not (Table 4); 4 patients with a follow-up of less than 3 months were excluded. Logistic regression analysis revealed an independent association between SERPINF1 rs12603825 and these subgroups for age, gender, smoking status, and GLD (P = 0.0027). We next conducted a survival analysis of the retreatment-free period in 150 PCV eyes that had been inactivated with a single PDT to evaluate whether this SNP was
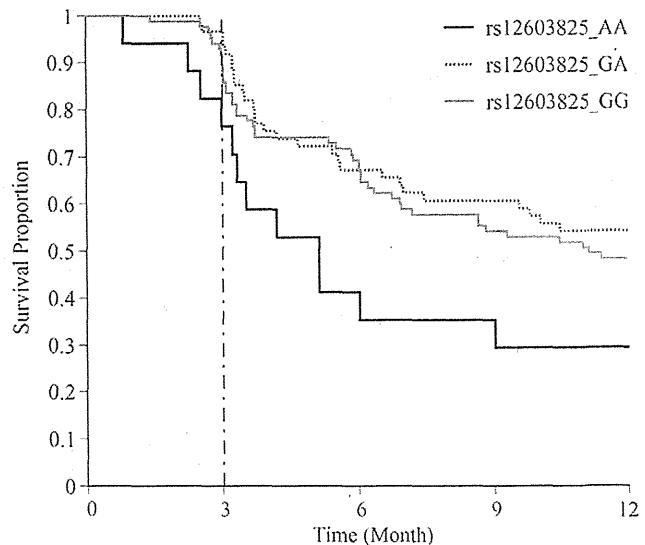

Figure 2. Overall survival analysis curve for the retreatment-free period among patients with the genotype of rs12603825. Patients with AA genotype were administered additional treatment after the first PDT within a significantly shorter period of time than those with other genotypes (P = 0.0038).

associated with recurrence of PCV, but there was no significant difference in the retreatment-free period among genotypes of rs12603825 (P = 0.36), even after adjusting the recessive model (P = 0.16) (Fig 4, available at http://aaojournal.org.).

Visual Outcomes

The visual outcomes after PDT were examined. Seventy-five patients from Kyoto University Hospital were followed up for more than 1 year after their first treatment. Although no significant difference in visual outcomes was observed in lesion size or smoking status (P = 0.523 and 0.468, respectively) (Fig 5, available at http://aaojournal.org.), visual outcomes of patients with the AA genotype of SERPINF1 rs12603825 were significantly worse than those with other genotypes (P = 0.013) (Fig 6).

Table 3. Association Results of Survival Analysis from Screening and Overall Genotyping

SNP	Chr*	Position*	Ref.[†]	Var.[†]	Gene*	Screening Sample (n 5 31)	All Sample (n 5 167)		
						Corrected P[§]	MAF	HWE P[‡]	Nominal P
rs17732513	14	91456132	C	T	FBLN5	0.000129	0.33	0.39	0.834
rs17793056	3	39284219	C	T	CX3CR1	0.00482	0.31	0.54	0.198
rs12603825	17	1620155	G	A	SERPINF1	0.000195	0.28	0.65	0.0117
rs12103559	17	1622128	G	A	SERPINF1	0.000107	–	–	–
rs1894286	17	1623659	C	T	SERPINF1	0.000162	–	–	–
rs11536889	9	119517952	G	C	TLR4	0.00021	0.23	0.24	0.733
Best-fitting model for significant results									
rs12603825	Recessive model								0.0038

HWE = Hardy–Weinberg equilibrium; MAF = minor allele frequency; SNP = single nucleotide polymorphism.
*Chromosome and position of markers refer to NCBI Build 36.1.
[†]Ref. and Var. are the reference and variant nucleotides, respectively, that are defined on the reference sequence of NCBI Build 36.1.
[‡]Hardy–Weinberg equilibrium for genotypic distribution was examined by the Hardy–Weinberg equilibrium exact test.
[§]P value corrected for multiple testing using the Bonferroni method.

Discussion

The present study found a significant association between the SERPINF1 gene variants and the clinical response of PCV to PDT; those patients who were homozygous for the minor allele A of SERPINF1 rs12603825 were administered an additional treatment within a significantly shorter period of time after the first PDT, were significantly less apt to be inactivated by a single treatment (independently of baseline clinical characteristics and smoking status), and had significantly worse visual acuity after PDT than those with no more than 1 copy of the minor allele.

SERPINF1 gene encodes serpin peptidase inhibitor, clade F, member 1, which is also referred to as pigment

Table 4. Clinical Characteristics and Genotype Distribution of the Study Population by Response to Single Photodynamic Therapy

	Photodynamic Therapy Less Effective*	Photodynamic Therapy Effective*	Adjusted P Value[†]
No. of patients	13	150	
Mean age (yrs)	69.92	72.19	0.222
Gender			0.283
Women	4 (30.8)	42 (28.0)	
Men	9 (69.2)	108 (72.0)	
Smoking history			0.489
Never	4 (36.4)	59 (41.3)	
Previous	4 (36.4)	55 (38.5)	
Current	3 (27.2)	29 (20.3)	
GLD			0.677
≤1800 μm	2 (15.4)	18 (12.4)	
1800–5400 μm	10 (76.9)	118 (81.4)	
>5400 μm	1 (7.7)	9 (6.2)	
SERPINF1_rs12603825		(GA+GG) vs. AA	0.0027
AA	4 (30.7)	10 (6.8)	
GA	2 (15.4)	60 (40.5)	
GG	7 (53.8)	78 (52.7)	

GLD = greatest linear dimension.
*Patients were divided into 2 subgroups by whether additional treatment was required within the first 3-month follow-up after a single PDT. Less effective = required; effective = not required.
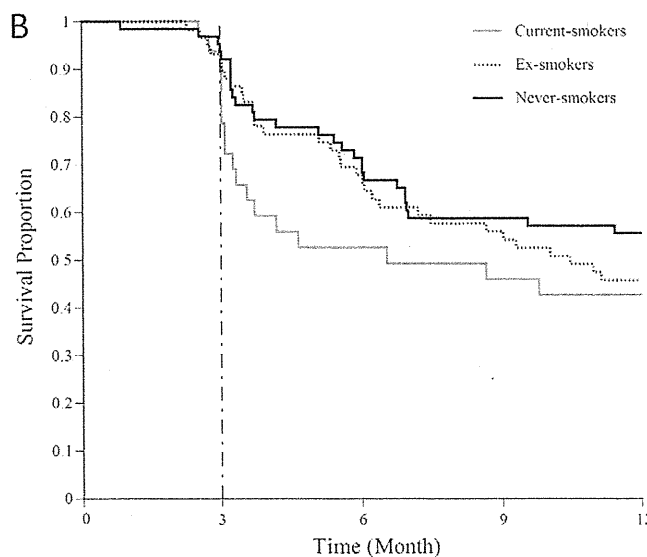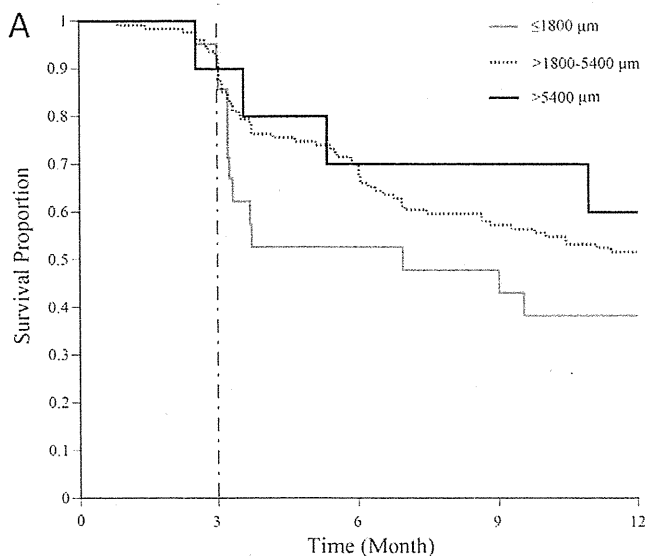[†]Adjusted for age, gender, smoking status, greatest linear dimension, and genotype.

epithelium-derived factor (PEDF), and was purified first from conditioned medium of human retinal pigment epithelial cells as a factor with potent neural differentiating activity.[27] Subsequent studies have revealed significantly reduced expression of PEDF in retinal pigment epithelial cells, Bruch's membrane,[28,29] and the vitreous[30] of eyes with AMD, whereas other studies have demonstrated the impact of PDT on the expression of PEDF.[31–33] By taking into consideration that PEDF inhibits the migration of endothelial cells in vitro and the in vivo development of experimental retinal neovascularization and choroidal

Figure 3. Overall survival analysis curve for the retreatment-free period by the 3 groups of GLD (**A**) and smoking status (**B**). There was no significant difference among these groups (P = 0.214 and 0.166, respectively), although borderline evidence of an association was observed between those who never smoked and ex-smokers plus current smokers (P = 0.060).
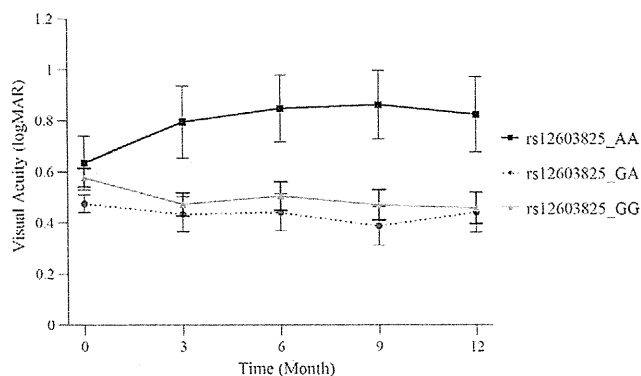

Figure 6. Visual prognosis by SERPINF1 rs12603825 after PDT. Visual outcomes of patients with the AA genotype were significantly worse than those with other genotypes (P = 0.013). Error bars represent ± 1 standard error of the mean. logMAR = logarithm of the minimum angle of resolution.

neovascularization,[34–36] the findings are consistent with those of the present study showing an association between the PEDF gene variants and the response to PDT.

The present study also shows the possibility that PEDF polymorphisms affect PEDF expression in eyes with PCV. We then evaluated whether rs12603825 affects PEDF gene expression in vivo using the raw data deposited in the Gene Expression Omnibus[37] as GSE 6536 (available at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6536, accessed July 1, 2010). However, there proved to be no association between SERPINF1 rs12603825 and PEDF gene expression ($P = 0.689$, analysis of variance test), and no significant differences in the baseline clinical characteristics among various genotypes of this SNP (Table 5, available at http://aaojournal.org.). Thus, the PEDF polymorphism may not result in the phenotypic difference via a change in PEDF gene expression. PEDF polymorphisms may influence the binding affinity to the receptor or indirectly affect PEDF expression after PDT by affecting the pathway between PDT and the PDT-induced change of PEDF expression.

As shown by the patients with rs12603825 AA genotype (Fig 1), another possibility regards PEDF polymorphisms as determinants of the probability of hemorrhage after PDT in eyes with PCV, which influences visual prognosis in the long term. All 3 patients with a macular hemorrhage among those who required additional treatment within 3 months after PDT had an rs12603825 AA genotype. Recurrent hemorrhage is one of the most symbolic signs of PCV,[19,21] and visual outcome is poor in eyes that have a massive subretinal hemorrhage involving the macula.[14,38] Furthermore, subretinal hemorrhage after PDT is a common finding in patients with PCV.[39,40] Yokoi et al[41] reported that PEDF levels in vitreous fluid were associated with vitreous hemorrhage in proliferative diabetic retinopathy, but the relationship between hemorrhage and PEDF is not fully understood. With this hypothesis in mind, our study may enhance our understanding of the mechanisms of hemorrhage in PCV.

Previous studies have shown the possibility that small lesions in patients with AMD respond better to PDT than larger lesions,[10,42] but the current study found no significant association between baseline lesion size and response to PDT. Conversely, we found that individuals who never smoked were less prone to recurrence than ex-smokers or current smokers. This result seems to be in agreement with the numerous studies that have shown smoking to be a risk factor for the development of AMD[43–46] and that smoking strongly influences the development of PCV.[18–20]

Study Limitations

One limitation of the present study is the number of participants. We found no significant association between rs12603825 and the retreatment-free period in eyes with PCV that responded to a single administration of PDT, although patients homozygous for the minor allele did tend to be administered additional treatment within a shorter period of time than those with other genotypes in the long term (Fig 4, available at http://aaojournal.org.). This tendency might reach statistical significance if the number of patients were increased. Other reports have demonstrated an association between the initial response and the risk of recurrence in other diseases.[47,48] Another limitation is the subgroup that initially responded to PDT may not represent a true difference in histologic response to PDT, because this relied on clinical information. Further basic research is needed to better characterize the relationship between the PEDF gene and the response to PDT. Another limitation is the absence of evaluation for the response to repeated treatments of PDT. Approximately half of the patients who noted less-effective responses to the first PDT received other treatments (e.g., anti-VEGF therapy or combined treatment) as their additional therapy. Further validation studies (e.g., prospective study) are obviously needed to clarify the detailed clinical response to PDT.

In conclusion, this study provides the first evidence that clinical, environmental, and genetic factors influence the response of PCV to PDT: PEDF gene variants associate independently with their response to PDT. Although it remains controversial as to whether PCV represents a subtype of neovascular AMD, the response to PDT is completely different for PCV and for neovascular AMD. Intravitreal injection of adenoviral vectors containing PEDF complementary DNA has been suggested to be a viable approach to therapy for neovascular AMD;[49,50] thus, our findings may lead to ways to modify the effects of PDT, to new methods of treatment using these materials, and to an understanding of the pathogenesis of PCV.

References

1. Sho K, Takahashi K, Yamada H, et al. Polypoidal choroidal vasculopathy: incidence, demographic features, and clinical characteristics. Arch Ophthalmol 2003;121:1392–6.
2. Maruko I, Iida T, Saito M, et al. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. Am J Ophthalmol 2007;144:15–22.
3. Ciardella AP, Donsoff IM, Huang SJ, et al. Polypoidal choroidal vasculopathy. Surv Ophthalmol 2004;49:25–37.
4. Edwards AO, Ritter R III, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. Science 2005;308:421–4.
5. Gotoh N, Nakanishi H, Hayashi H, et al. *ARMS2 (LOC387715)* variants in Japanese patients with exudative age-related macular degeneration and polypoidal choroidal vasculopathy. Am J Ophthalmol 2009;147:1037–41.
6. Gotoh N, Yamada R, Nakanishi H, et al. Correlation between *CFH* Y402H and *HTRA1* rs11200638 genotype to typical exudative age-related macular degeneration and polypoidal choroidal vasculopathy phenotype in the Japanese population. Clin Experiment Ophthalmol 2008;36:437–42.
7. Gotoh N, Kuroiwa S, Kikuchi T, et al. Apolipoprotein E polymorphisms in Japanese patients with polypoidal choroidal vasculopathy and exudative age-related macular degeneration. Am J Ophthalmol 2004;138:567–73.
8. Seddon JM, Reynolds R, Maller J, et al. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. Invest Ophthalmol Vis Sci 2009;50:2044–53.
9. Treatment of Age-Related Macular Degeneration With Photodynamic Therapy Study Group. Verteporfin therapy of

subfoveal choroidal neovascularization in patients with age-related macular degeneration: additional information regarding baseline lesion composition's impact on vision outcomes-TAP report no. 3. Arch Ophthalmol 2002;120: 1443–54.

10. Arias L, Pujol O, Berniell J, et al. Impact of lesion size on photodynamic therapy with verteporfin of predominantly classic lesions in age related macular degeneration. Br J Ophthalmol 2005;89:312–5.

11. Immonen I, Seitsonen S, Tommila P, et al. Vascular endothelial growth factor gene variation and the response to photodynamic therapy in age-related macular degeneration. Ophthalmology 2010;117:103–8.

12. Feng X, Xiao J, Longville B, et al. Complement factor H Y402H and C-reactive protein polymorphism and photodynamic therapy response in age-related macular degeneration. Ophthalmology 2009;116:1908–12.

13. Spaide RF, Donsoff I, Lam DL, et al. Treatment of polypoidal choroidal vasculopathy with photodynamic therapy. Retina 2002;22:529–35.

14. Gomi F, Ohji M, Sayanagi K, et al. One-year outcomes of photodynamic therapy in age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. Ophthalmology 2008;115:141–6.

15. Tsuchiya D, Yamamoto T, Kawasaki R, Yamashita H. Two-year visual outcomes after photodynamic therapy in age-related macular degeneration patients with or without polypoidal choroidal vasculopathy lesions. Retina 2009;29:960–5.

16. Yamashiro K, Tsujikawa A, Nishida A, et al. Recurrence of polypoidal choroidal vasculopathy after photodynamic therapy. Jpn J Ophthalmol 2008;52:457–62.

17. Kurashige Y, Otani A, Sasahara M, et al. Two-year results of photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol 2008;146:513–9.

18. Kikuchi M, Nakamura M, Ishikawa K, et al. Elevated C-reactive protein levels in patients with polypoidal choroidal vasculopathy and patients with neovascular age-related macular degeneration. Ophthalmology 2007;114:1722–7.

19. Laude A, Cackett PD, Vithana EN, et al. Polypoidal choroidal vasculopathy and neovascular age-related macular degeneration: same or different disease? Prog Retin Eye Res 2010;29: 19–29.

20. Nakanishi H, Yamashiro K, Yamada R, et al. Joint effect of cigarette smoking, *CFH* and *LOC387715/HTRA1* polymorphisms on polypoidal choroidal vasculopathy. Invest Ophthalmol Vis Sci 2010;51:6183–7. Epub 2010 Aug 4.

21. Japanese Study Group of Polypoidal Choroidal Vasculopathy. Criteria for diagnosis of polypoidal choroidal vasculopathy [in Japanese]. Nippon Ganka Gakkai Zasshi 2005;109:417–27.

22. Otani A, Sasahara M, Yodoi Y, et al. Indocyanine green angiography: guided photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol 2007;144:7–14.

23. Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: one-year results of 2 randomized clinical trials—TAP report 1. Arch Ophthalmol 1999;117:1329–45.

24. Tano Y, Ophthalmic PDT Study Group. Guidelines for PDT in Japan [letter]. Ophthalmology 2008;115:585.

25. Chan WM, Lam DS, Lai TY, et al. Photodynamic therapy with verteporfin for symptomatic polypoidal choroidal vasculopathy: one-year results of a prospective case series. Ophthalmology 2004;111:1576–84.

26. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263–5.

27. Tombran-Tink J, Chader GG, Johnson LV. PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity [letter]. Exp Eye Res 1991;53:411–4.

28. Bhutto IA, Uno K, Merges C, et al. Reduction of endogenous angiogenesis inhibitors in Bruch's membrane of the submacular region in eyes with age-related macular degeneration. Arch Ophthalmol 2008;126:670–8.

29. Bhutto IA, McLeod DS, Hasegawa T, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. Exp Eye Res 2006;82:99–110.

30. Holekamp NM, Bouck N, Volpert O. Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration. Am J Ophthalmol 2002;134:220–7.

31. Schmidt-Erfurth U, Schlotzer-Schrehard U, Cursiefen C, et al. Influence of photodynamic therapy on expression of vascular endothelial growth factor (VEGF), VEGF receptor 3, and pigment epithelium-derived factor. Invest Ophthalmol Vis Sci 2003;44:4473–80.

32. Tatar O, Adam A, Shinoda K, et al. Expression of VEGF and PEDF in choroidal neovascular membranes following verteporfin photodynamic therapy. Am J Ophthalmol 2006;142:95–104.

33. Obata R, Iriyama A, Inoue Y, et al. Triamcinolone acetonide suppresses early proangiogenic response in retinal pigment epithelial cells after photodynamic therapy in vitro. Br J Ophthalmol 2007;91:100–4.

34. Dawson DW, Volpert OV, Gillis P, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 1999;285:245–8.

35. Mori K, Duh E, Gehlbach P, et al. Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. J Cell Physiol 2001;188:253–63.

36. Mori K, Gehlbach P, Yamamoto S, et al. AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization. Invest Ophthalmol Vis Sci 2002;43: 1994–2000.

37. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30:207–10.

38. Jalali S, Parra SL, Majji AB, et al. Ultrasonographic characteristics and treatment outcomes of surgery for vitreous hemorrhage in idiopathic polypoidal choroidal vasculopathy. Am J Ophthalmol 2006;142:608–19.

39. Hirami Y, Tsujikawa A, Otani A, et al. Hemorrhagic complications after photodynamic therapy for polypoidal choroidal vasculopathy. Retina 2007;27:335–41.

40. Ojima Y, Tsujikawa A, Otani A, et al. Recurrent bleeding after photodynamic therapy in polypoidal choroidal vasculopathy. Am J Ophthalmol 2006;141:958–60.

41. Yokoi M, Yamagishi S, Saito A, et al. Positive association of pigment epithelium-derived factor with total antioxidant capacity in the vitreous fluid of patients with proliferative diabetic retinopathy. Br J Ophthalmol 2007;91:885–7.

42. Verteporfin In Photodynamic Therapy Study Group. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization—Verteporfin in Photodynamic Therapy report 2. Am J Ophthalmol 2001;131: 541–60.

43. Eye Disease Case-Control Study Group. Risk factors for neo-vascular age-related macular degeneration. Arch Ophthalmol 1992;110:1701–8.
44. Christen WG, Glynn RJ, Manson JE, et al. A prospective study of cigarette smoking and risk of age-related macular degeneration in men. JAMA 1996;276:1147–51.
45. Seddon JM, Willett WC, Speizer FE, Hankinson SE. A prospective study of cigarette smoking and age-related macular degeneration in women. JAMA 1996;276:1141–6.
46. Hyman LG, Lilienfeld AM, Ferris FL III, Fine SL. Senile macular degeneration: a case-control study. Am J Epidemiol 1983;118:213–27.
47. Huber KE, Carey LA, Wazer DE. Breast cancer molecular subtypes in patients with locally advanced disease: impact on prognosis, patterns of recurrence, and response to therapy. Semin Radiat Oncol 2009;19:204–10.
48. Van den Eynde E, Tiraboschi JM, Tural C, et al. Ability of treatment week 12 viral response to predict long-term outcome in genotype 1 hepatitis C virus/HIV coinfected patients. AIDS 2010;24:975–82.
49. Campochiaro PA, Nguyen QD, Shah SM, et al. Adenoviral vector-delivered pigment epithelium-derived factor for neo-vascular age-related macular degeneration: results of a phase I clinical trial. Hum Gene Ther 2006;17:167–76.
50. Imai D, Yoneya S, Gehlbach PL, et al. Intraocular gene transfer of pigment epithelium-derived factor rescues photo-receptors from light-induced cell death. J Cell Physiol 2005; 202:570–8.

Footnotes and Financial Disclosures

[1] Department of Ophthalmology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

[2] Center for Genomic Medicine/Inserm U.852, Kyoto University Graduate School of Medicine, Kyoto, Japan.

[3] Department of Ophthalmology, Fukushima Medical University, Fukushima, Japan.

[4] Department of Ophthalmology, Kobe City Medical Center General Hospital, Kobe, Japan.

Correspondence:
Kenji Yamashiro, MD, PhD, Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, 54 Kawahara, Shogoin, Sakyo, Kyoto 606-8507, Japan. E-mail: yamashiro@kuhp.kyoto-u.ac.jp.

Association of 15q14 and 15q25 with High Myopia in Japanese

Hisako Hayashi,[1,2] Kenji Yamashiro,[1] Hideo Nakanishi,[1,2] Isao Nakata,[1,2] Yumiko Kurashige,[1,2] Akitaka Tsujikawa,[1] Muka Moriyama,[3] Kyoko Ohno-Matsui,[3] Manabu Mochizuki,[3] Mineo Ozaki,[4] Ryo Yamada,[2] Fumihiko Matsuda,[2] and Nagahisa Yoshimura[1]

PURPOSE. To investigate whether there are associations of genetic variations in chromosome 15q14 and 15q25, recently shown to confer risk of refractive error and myopia in Caucasians, with high myopia in Japanese.

METHODS. A total of 1125 unrelated Japanese patients with high myopia and two independent control groups were evaluated (366 cataract patients without high myopia and 929 healthy Japanese patients). The four single nucleotide polymorphisms (SNPs) rs634990 and rs524952 at 15q14 and rs8027411 and rs17175798 at 15q25 were genotyped.

RESULTS. A significant association with high myopia was observed in 15q14 ($P = 0.0035$ for rs634990 and $P = 0.0017$ for rs524952 when evaluated with cataract controls and $P = 1.91 \times 10^{-6}$ for rs634990 and $P = 8.78 \times 10^{-7}$ for rs524952 with healthy Japanese controls). When evaluated with cataract controls, the odds ratios (95% confidence intervals) were 1.30 (1.10–1.53) for rs634990 C allele and 1.32 (1.11–1.56) for rs524952 A allele. The population attributable risks were 0.29 and 0.30, respectively. The SNPs in 15q25 did not show a significant association with high myopia when evaluated with cataract control ($P > 0.42$), while it showed a weak association when evaluated with healthy Japanese controls ($P = 0.031$ for rs8027411 and $P = 0.047$ for rs17175798) with odds ratios of 1.17 (1.03–1.33) for rs8027411 T allele and 1.15 (1.02–1.31) for rs17175798 C allele.

CONCLUSIONS. These findings suggest that a region in 15q14 is susceptibility loci for high myopia. This locus harbor susceptibility genes for not only common myopia but also for high myopia. The 15q25 locus might also have association to

myopia. (*Invest Ophthalmol Vis Sci.* 2011;52:4853–4858) DOI:10.1167/iovs.11-7311

Myopia is the most common visual disorder in the world, with the prevalence estimated to be 25% in the United States and Western Europe and to be much higher (40%–70%) in Asians, including Chinese and Japanese.[1-5] Myopic eyes with very long axial lengths (≥ 26 mm) or a high degree of myopic refractive error (≤ -6 D) are classified as high myopia.[6] Recently, the prevalence of high myopia has been increasing worldwide, especially in the younger East Asian population.[7,8] It is well known that high myopia is associated with various ocular complications,[9] and it is one of the leading causes of legal blindness in developed countries.[10-12] Therefore, it is important to develop methods for preventing or delaying the onset of high myopia or for limiting its progression.

The cause of myopia is not fully understood, and, in fact, it is not yet clear that common myopia and high myopia share the same background; high myopia may have a unique background that distinguishes it from common myopia. It has been shown that environmental factors (in particular near work, a higher level of education, and less time spent in outdoor activities) and genetic factors contribute to the development of myopia.[13] Twin studies provide compelling evidence that myopia is inherited,[14,15] and multiple family-based whole genome linkage analysis indicate several heritable myopia susceptibility loci, such as the MYP1-18 loci.[16-31] Some of these loci are reported to be associated with common myopia, high myopia, or both. However, several studies could not replicate these associations.[32-34] In addition, many candidate genes associated with high myopia have been reported.[35-43] However, most of these associated candidate genes have been negated by subsequent studies, and no genes have yet been identified that are consistently responsible for either common or high myopia.

Genome-wide association studies (GWAS) are expected to reveal the susceptibility genes of many complex diseases, as shown in age-related macular degeneration (AMD). We performed the first genome-wide association study for high myopia and showed that several single nucleotide polymorphisms (SNPs) at 11q24.1 are associated significantly with high myopia in Japanese.[44] However, a study in Han Chinese did not show the association of rs577948, one of the SNPs, with high myopia.[45]

Recently, two other groups have performed GWAS for refractive error and showed that SNPs in 15q14 and 15q25 are associated with refractive error and myopia,[46,47] but the cohorts used in these two studies were population-based, and only a limited number (1.7%–4.0%) of patients with high myopia were included. Although it is not clear if common and high myopia share the same genetic background, some MYP loci are reported to be associated with both common myopia

and high myopia. In this study, we evaluated the associations of reported SNPs with high myopia in Japanese.

Moreover, we investigated their associations with the occurrence of choroidal neovascularization (CNV) in high myopia. Some highly myopic eyes develop CNV, while other highly myopic eyes do not, and because CNV is one of the most vision-threatening complications in highly myopic eyes, the investigation of the mechanisms of how it occurs is important. We evaluated the influence of susceptibility genes for neovascular AMD on the occurrence of CNV in myopic eyes and found that these genes do not affect the development of myopic CNV.[48] Axial elongation of highly myopic eyes results in thinning of the retina and choroid, patchy chorioretinal atrophy, and lacquer cracks, all of which are important predisposing conditions for the development of CNV.[49,50] It could be hypothesized that CNV occurs when the eye is strongly affected by susceptibility genes for myopia, or it may be that specific susceptibility genes exist for myopic CNV—genes that are in addition to the susceptibility genes for myopia.

MATERIALS AND METHODS

All procedures in this study adhered to the tenets of the Declaration of Helsinki. The institutional review boards and the ethics committees of each institution involved approved the protocols of this study. All patients were fully informed of the purpose and procedures of this study, and written consent was obtained from each patients.

Patients

One thousand one hundred twenty-five unrelated highly myopic Japanese patients were recruited from Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Ozaki Eye Hospital, and Otsu Red-cross Hospital (mean age ± SD, 57.6 ± 14.8 years; male/female ratio, 33.5%/66.5%). All patients had a comprehensive ophthalmic examination, including dilated indirect and contact lens slit lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA). The difference between these two devices would be around 0.1 mm.[52] When a patient who visited the aforementioned hospitals had an axial length of >26.1 mm in both eyes, peripheral blood was obtained after informed consent and used as high-myopia patients group. To evaluate the contribution of the SNPs between high myopic patients with CNV and high myopic patients without CNV, the high myopic patients group was separated into a CNV group (600 patients) and a no CNV

group (450 patients). Inclusion criteria of the CNV group were clinical presentation and angiographic manifestations of macular CNV or Fuchs' spot in at least one eye.

As the control subjects, we used 366 cataract patients with axial lengths <25.0 mm in both eyes (control 1). These patients were recruited from the Department of Ophthalmology at Kyoto University Hospital, the Ozaki Eye Hospital, the Japanese Red Cross Otsu Hospital, and Nagahama City Hospital; their mean age (± SD) was 74.4 ± 8.4 years, and there were 39.9% men and 60.1% women. The axial length was measured with applanation A-scan ultrasonography or partial coherence interferometry before cataract surgery and dilated fundus examination was performed after surgery. If the fundus examination revealed myopic change such as lacquer cracks/peripapillary atrophy, staphyloma or CNV, the subject was eliminated from the control 1 group.

We also used DNA samples from 929 subjects who were randomly selected from the Pharma SNP Consortium (PSC); this constituted control group 2. This group has been used for previous genomic studies and is regarded as being representative of the general Japanese population (mean age ± SD, 38.8 ± 11.8 years; male/female ratio, 61.7%/38.3%).[51] All were Japanese and none had any history of ocular disease.

Genotyping and Statistical Analyses

Genomic DNAs were prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). Four SNPs (rs634990, rs524952, rs8027411, and rs17175798) were selected based on their specific presence in two previous GWAS; they were genotyped using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). Deviations in genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed for each group with the HWE exact test. The χ^2 test for trend or its exact counterpart was used to compare the genotype distributions of two groups. To adjust age and sex, we performed multiple regression and logistic regression analysis. These statistical analyses were performed with R software R (R Foundation for Statistical Computing, Vienna, Austria, available at http://www.rproject.org/). $P < 0.05$ was considered statistically significant.

RESULTS

The demographics of the study population are shown in Table 1. The axial length of the 2258 eyes of the 1129 high myopia cases ranged from 26.11 to 39.73 mm, with a mean ± SD of

TABLE 1. Characteristics of the Study Population

	Patients	Controls	
	High Myopia*	Cataract†	PSC‡
Patients, *n*	1125	366	929
Age in years, Mean ± SD	57.57 ± 14.75	74.40 ± 8.37	38.81 ± 11.83
Sex, *n* (%)			
Male	377 (33.5%)	146 (39.9%)	573 (61.7%)
Female	748 (66.5%)	220 (60.1%)	356 (38.3%)
Axial length, mm ± SD			
Right eyes	29.18 ± 1.95	23.05 ± 1.00	NA
Left eyes	29.01 ± 2.16	23.01 ± 1.66	NA
Refraction of the phakic eyes, D§			
Right eyes	−10.79 ± 6.96	−0.23 ± 2.56	NA
Left eyes	−10.36 ± 5.82	−0.11 ± 2.52	NA

* Axial length of >26.1 mm in both eyes.

† Individuals who underwent cataract surgery and who had an axial length <25.00 mm in both eyes.

‡ DNA samples randomly selected from the Pharma SNP Consortium (PSC).

§ For calculations of refraction, eyes that had undergone cataract surgery or corneal refractive surgery were excluded.

TABLE 2. Genotype Counts, Associations, and Odds Ratios in the High Myopia Patients and Cataract Controls

Locus	SNP ID		Genotype		P*	Adjusted P†	OR (95% CI)	PAR
			High Myopia	Controls				
15q14	rs634990 (C/T)	CC	304	84	0.0026	0.0035	1.65 (1.19–2.29)	0.29
		CT	571	165			1.58 (1.19–2.09)	
		TT	246	112			1.00 (ref)	
	rs524952 (A/T)	AA	303	81	0.0015	0.0017	1.70 (1.22–2.37)	0.30
		AT	572	164			1.59 (1.19–2.11)	
		TT	244	111			1.00 (ref)	
15q25	rs8027411 (G/T)	TT	428	116	0.17	0.42	0.87 (0.60–1.26)	0.03
		GT	525	193			1.17 (0.82–1.67)	
		GG	166	52			1.00 (ref)	
	rs17175798 (C/T)	CC	422	117	0.25	0.60	1.12 (0.77–1.62)	0.04
		CT	528	193			0.85 (0.60–1.20)	
		TT	171	53			1.00 (ref)	

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; PAR, population attributable risk.

* Differences in the observed genotypic distribution were examined by χ^2 test for trend.

† Age and sex adjustment was performed based on a logistic regression model.

29.09 ± 2.04 mm. Among the 2250 eyes enrolled, 1753 (77.9%) were phakic, 427 (19.0%) were pseudophakic, and 70 (3.1%) were aphakic. The mean refraction of the 1753 phakic eyes was −10.52 ± 6.71 D. The axial length of the 732 eyes of the cataract patients ranged from 18.67 to 24.89 mm, with a mean ± SD of 23.02 ± 1.28 mm. Mean refraction of the phakic eyes in control group 1 was −0.14 ± 2.2 D.

Genotype counts, associations examined with χ^2 test for trend, and odds ratios for the four SNPs between the high myopia patients and the controls are shown in Tables 2 and 3. The distributions of the genotypes for the four SNPs were all in HWE ($P > 0.1$), as assessed with the exact test. Rs634990 and rs524952 were in almost complete linkage disequilibrium, as were rs8027411 and rs17175798 (D' > 0.95).

When evaluated with control group 1 (cataract patient controls, $n = 366$), the SNPs rs634990 and rs524952 in 15q14 showed a significant association with high myopia ($P = 0.0035$ and 0.0017, respectively). The odds ratios (95% confidence intervals [CIs]) were 1.30 (1.10–1.53) for rs634990 C allele and 1.32 (1.11–1.56) for rs524952 A allele. When evaluated with control group 2 (healthy Japanese controls, $n = 929$), these two SNPs showed strong association with high myopia ($P = 2.21 \times 10^{-6}$ and 1.62×10^{-6}, respectively). The odds ratios were 1.36 (1.20–1.54) for rs634990 C allele and 1.37 (1.21–

1.55) for rs524952 A allele. The population attributable risk (PAR) was 0.29 for rs634990 and 0.30 for rs524952 when evaluated with both controls, and the risk alleles were the same as those reported previously.

The variants in 15q25 (rs8027411 and rs17175798) showed no association to high myopia when evaluated with control group 1 ($n = 366$), while these two SNPs showed marginal associations ($P = 0.031$ and 0.047, respectively) when evaluated with control group 2 ($n = 929$). The minor allele frequencies in these two controls were very similar; 0.41 for both SNPs in control group 1 and 0.42 for both SNPs in control group 2, and the risk alleles seemed to be the same as those reported previously. The odds ratios were 1.13 (0.95–1.34) for rs8027411 T allele and 1.10 (0.93–1.31) for rs17175798 C allele when evaluated with control group 1 and 1.17 (1.03–1.33) and 1.15 (1.02–1.31), respectively, when evaluated with control group 2. The PARs were 0.03 for rs8027411 and 0.04 for rs17175798 when evaluated with control group 1 and 0.17 and 0.14, respectively, when evaluated with control group 2.

Associations for the four SNPs with CNV in highly myopic eyes of the Japanese population in this study are shown in Table 4. We analyzed the genotype distributions for rs634990, rs524952, rs8027411, and rs17175798 in high myopia patients with CNV and compared them with high myopia patients

TABLE 3. Genotype Counts, Associations, and Odds Ratios in the High Myopia Patients and Pharma SNP Consortium Controls

Locus	SNP ID		Genotype		P*	Adjusted P†	OR (95% CI)	PAR
			High Myopia	Controls				
15q14	rs634990 (C/T)	CC	304	191	1.1×10^{-6}	1.91×10^{-6}	1.84 (1.44–2.36)	0.29
		CT	571	442			1.50 (1.21–1.85)	
		TT	246	285			1.00 (ref)	
	rs524952 (A/T)	AA	303	191	7.60×10^{-7}	8.78×10^{-7}	1.86 (1.45–2.39)	0.30
		AT	572	444			1.51 (1.22–1.86)	
		TT	244	286			1.00 (ref)	
15q25	rs8027411 (G/T)	TT	428	310	0.013	0.031	1.37 (1.06–1.78)	0.17
		GT	525	445			1.17 (0.91–1.51)	
		GG	166	165			1.00 (ref)	
	rs17175798 (C/T)	CC	422	309	0.026	0.047	1.32 (1.02–1.71)	0.14
		CT	528	453			1.12 (0.88–1.44)	
		TT	171	165			1.00 (ref)	

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; PAR, population attributable risk.

* Differences in the observed genotypic distribution were examined by χ^2 test for trend.

† Age and sex adjustment was performed based on a logistic regression model.