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REPORTING VISUAL ACUITIES

The AJO encourages authors to report the visual acuity in the manuscript using the same nomenclature that was used in gathering the data provided they were recorded in one of the methods listed here. This table of equivalent visual acuities is provided to the readers as an aid to interpret visual acuity findings in familiar units.

Table of Equivalent Visual Acuity Measurements

Snellen Visual Acuities				
4 Meters	6 Meters	20 Feet	Decimal Fraction	LogMAR
4/40	6/60	20/200	0.10	+1.0
4/32	6/48	20/160	0.125	+0.9
4/25	6/38	20/125	0.16	+0.8
4/20	6/30	20/100	0.20	+0.7
4/16	6/24	20/80	0.25	+0.6
4/12.6	6/20	20/63	0.32	+0.5
4/10	6/15	20/50	0.40	+0.4
4/8	6/12	20/40	0.50	+0.3
4/6.3	6/10	20/32	0.63	+0.2
4/5	6/7.5	20/25	0.80	+0.1
4/4	6/6	20/20	1.00	0.0
4/3.2	6/5	20/16	1.25	−0.1
4/2.5	6/3.75	20/12.5	1.60	−0.2
4/2	6/3	20/10	2.00	−0.3

From Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982;94:91–96.

Biosketch

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CLINICAL INVESTIGATION

VEGF gene polymorphism and response to intravitreal bevacizumab and triple therapy in age-related macular degeneration

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Abstract
Purpose To investigate the association between the vascular endothelial growth factor (VEGF) gene and response to either intravitreal bevacizumab (IVB) or photodynamic therapy with intravitreal triamcinolone acetonide and IVB (triple therapy) for neovascular age-related macular degeneration (AMD).
Methods The study consisted of 94 patients with neovascular AMD who underwent IVB and 79 patients with neovascular AMD who underwent triple therapy. Genotypes were determined for four selected tagging single-nucleotide polymorphism (SNP)s of the VEGF gene.
Results Of the four SNPs studied, one SNP (rs699946) was associated significantly with visual acuity (VA) changes 12 months after treatment—irrespective of whether they received IVB alone ($P = 0.044$) or triple therapy 0.010). Baseline VA was not significantly different among the three genotypes of rs699946 in either treatment group. There were no significant differences in the number of treatments, incidence of recurrence, or the period until the recurrence according to VEGF rs699946 genetic variant.
Conclusions The VEGF gene SNP rs699946 was associated with response to IVB alone and to triple therapy in this study. The G allele in SNP rs699946 can thus be applied as a marker for better visual prognosis in patients with neovascular AMD who receive either IVB or triple therapy.

Keywords Bevacizumab · Photodynamic therapy · Vascular endothelial growth factor

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Introduction

Age-related macular degeneration (AMD) is the leading cause of severe impairment of the visual function in people 50 years of age or older who reside in industrialized countries [1]. Recent treatment for neovascular AMD includes photodynamic therapy (PDT) [2] and anti-vascular endothelial growth factor (VEGF) therapy [3–8]. Because VEGF is expressed in choroidal neovascularization (CNV) [9–11] and induces an exudative change in the retina, anti-VEGF drugs can inactivate CNV and reduce the retinal exudative change. In addition to their direct role as a treatment for AMD, anti-VEGF drugs have been used in combination with PDT to suppress the VEGF upregulation that is typically induced by PDT. Recently, triple therapy using PDT combined with intravitreal bevacizumab (IVB) and intravitreal triamcinolone acetonide (IVTA) was introduced [12, 13].

VEGF is a major molecular mediator of neovascularization and of vascular permeability [14], and its expression is upregulated under hypoxic conditions. The VEGF gene is located on chromosome 6p12, and the coding region spans approximately 16.3 kb. Polymorphisms of the VEGF gene have been shown to be associated with levels of VEGF in both serum [15–17] and vitreous [18]. VEGF polymorphisms are associated with the prognosis of several diseases associated with VEGF [19–24]. Recently, Immonen et al. [25] demonstrated that VEGF

gene polymorphisms affect the anatomic outcome of AMD after PDT. However, the association of VEGF gene polymorphism with the visual prognosis of patients with AMD has not been examined and, despite the anatomic success after PDT, fibrosis can attenuate the relatively good visual outcomes, just as residual lesions of serous detachment after PDT do not always result in lower visual acuity (VA). It would thus be important to know exactly what factor(s) may determine the visual prognosis of eyes with AMD that have undergone treatment.

Because IVB treatment and triple therapy include anti-VEGF drugs, it is conceivable that VEGF gene polymorphism correlates with the results of this AMD treatment. In this study we sought to determine if there is any association between VEGF genotype and the response of neovascular AMD to IVB or to triple therapy.

Materials and methods

Patients and methods

This study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Institutional Review Board/Ethics Committee of Kyoto University Graduate School of Medicine, Kyoto, Japan. All patients provided written informed consent after the risks and benefits of the treatment were fully explained to them. For this retrospective study, we reviewed the medical records of 173 patients with neovascular AMD who had either received IVB alone (IVB group) or PDT combined with IVB and IVTA (triple-therapy group) at Kyoto University Hospital between May 2006 and November 2008. All patients included in the study had subfoveal CNV associated with AMD, VA better than 1.0, as the logarithm of the minimum angle of resolution (logMAR) at baseline, and were 50 years of age or older; patients were excluded if they had undergone surgical treatment for their AMD before either the IVB or triple therapy.

Before treatment, each patient had undergone a comprehensive ophthalmologic examination, including measurement of best-corrected VA, intraocular pressure testing, indirect ophthalmoscopy, and slitlamp biomicroscopy with a contact lens. Best-corrected VA was measured with a Landolt chart and converted to a logMAR. After fundus photographs were taken, fluorescein and indocyanine green angiography were performed and the retinal cross section was examined with optical coherence tomography (OCT). Diagnosis of polypoidal choroidal vasculopathy (PCV) was based on indocyanine green angiography, which showed a branching vascular network terminating in polypoidal swelling.

The IVB group had an intravitreal injection of 1.25 mg bevacizumab one to three times as the initial treatment. The second and/or third injections were performed at 1-month intervals, when angiography and/or OCT examination showed remnant macular exudative changes (serous retinal detachment or retinal edema on OCTs or angiographic leakage seen on FAs). For the patients in the triple-therapy group, 1.25 mg bevacizumab and 2 mg TA were administered intravitreally 3 to 4 days before the PDT. The treatment decisions were made by retina specialists at the Department of Ophthalmology at Kyoto University Hospital, who were unaware of the genotype of the patients. Retreatment was performed when an eye showed severe leakage of fluorescein dye during the late phase of fluorescein angiography or serous retinal detachment because of residual active CNV as seen on OCT usually after a 3-month interval. In the IVB group, an intravitreal anti-VEGF drug was given when recurrence was noted. During the sequence of monthly injections for initial treatment, we did not encounter any recurrence of exudative changes. In the triple-therapy group, PDT either with or without an intravitreal anti-VEGF drug or anti-VEGF treatment was performed for any recurrence that occurred.

Single nucleotide polymorphism selection and Genotyping

Four tagging VEGF single-nucleotide polymorphism (SNP)s were selected for investigation—two SNPs on the promoter region, rs699946 and rs699947, and two intronic SNPs, rs3025033 and 3025035. The four tagging VEGF SNPs provided 100% coverage for all common (minor allele frequency of more than 20%) HapMap [26] SNPs within a 26.3 kilobase region (16.3-kb gene length; 10 kb upstream) spanning the VEGF gene on chromosome 6 (r^2 threshold of 0.95). Genomic DNAs were prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). Genotyping was performed using the Taqman SNP assay with the ABI Prism 7700 system (Applied Biosystems, Foster City, CA, USA).

Data analysis

Descriptive statistics for all demographic and clinical variables were calculated and comparisons made using the unpaired t test for means with continuous data (e.g., age) and the chi-squared test for categorical data (e.g., gender). Visual acuity before and after treatment were compared among genotypes by use of analysis of variance (ANOVA) test and VA changes among genotypes were evaluated by use of the Jonckheere–Terpstra trend test, which is a

nonparametric test for ordered differences among classes [27]. Significance was defined at the 5% level.

Results

Patients with neovascular AMD who underwent treatment with IVB alone ($n = 94$) or with triple therapy ($n = 79$) were enrolled in this study. Demographic characteristics of the two groups are listed in Table 1. The mean baseline VA in the IVB group was significantly better than that in the triple-therapy group ($P = 0.038$). Seventy-five patients in the IVB group and 66 patients in the triple-therapy group were followed up for more than a year after their first treatment. The mean follow-up period for the IVB group was 13.1 months (range 3.0–35.3 months); that for the triple-therapy group was 13.1 months (range 3.0–22.7 months). The mean number of treatments during the 1-year follow-up period was 2.59 (range 1–8) in the IVB group and 1.61 (range 1–5) in the triple-therapy group—the number of treatments in the triple-therapy group being significantly less than that in the IVB group (P\ 0.01).

Figure 1 shows the mean VA changes from baseline for the IVB group and for the triple-therapy group. Members of both groups maintained vision—the mean change at 12 months from baseline being a gain of 0.026 for both groups. Although the VA in the IVB group tended to improve immediately after treatment, it had a tendency to improve more gradually in the triple-therapy group.

The genotype frequencies of the four VEGF tagging SNPs in each group are shown in Table 2. Although we chose SNPs with relatively high minor allele frequencies, there were only a few patients with the GG genotype in rs3025033 or the TT genotype in rs3025035 in the triple-therapy group; accordingly, we excluded these two SNPs from the analyses.

We examined first the differences in initial VA among the genotypes of rs699946 in the IVB and triple-therapy groups. Table 3 shows demographic characteristics by genotypes of rs699946 in each group. There was no significant difference in mean baseline VA among AA, AG, and GG genotypes in either group ($P = 0.101$ and 0.433, respectively). We evaluated next the changes in VA after treatment in each group; these changes are shown in Fig. 2. As depicted in this figure, the VA seemed to improve continuously in the patients with the GG genotype, whereas it did not change appreciably during the follow-up period in the patients with the AG or AA genotypes in either treatment group. When the mean VA at 12 months was examined, it was significantly different among the three genotypes in both treatment groups ($P = 0.025$ and 0.010, respectively). Furthermore, when we examined the trend in VA changes at 12 months among these three genotypes, the G allele showed a significant trend toward better visual acuity changes in both the IVB group ($P = 0.044$) and the triple-therapy group ($P = 0.010$). Visual acuity changes at 12 months were best in those with the GG genotype,

Table 1 Characteristics of the study population

	Bevacizumab	Triple therapy	*P* value
Number of patients	94	79	–
Mean age (years)	74.9	75.5	0.60
Female (%)	31.9%	26.6%	0.26
Right eye (%)	50.0%	46.8%	0.42
Mean baseline VA	0.546	0.631	0.038
Mean follow-up (months)	13.1	13.1	0.87

VA visual acuity

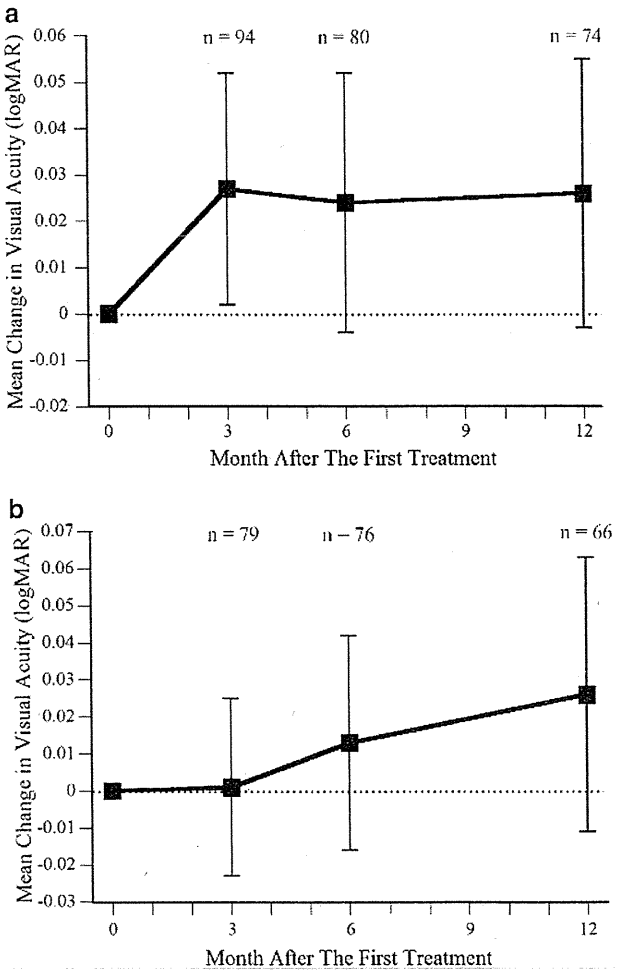

Fig. 1 Visual acuity over time for the IVB and triple-therapy groups. **a** Visual acuity (VA) over time in patients treated with intravitreal bevacizumab alone (IVB group) and, **b** in patients treated with photodynamic therapy with IVB and intravitreal triamcinolone acetonide (triple-therapy group) who were followed up for more than a year after their first treatment. Both show the mean VA change ± SEM in logMAR from baseline

middle in those with the GA genotype, and worst in those with the AA genotype. We evaluated whether rs699946 is also associated with visual prognosis after the treatment in the subgroups of PCV and typical AMD. We found the same tendency for the visual prognosis in both subgroups, although most of the analyses did not reach a significant level because of the small sample size (data not shown).

Next, we evaluated whether there were significant differences in the number of treatments, incidences of recurrence, or the periods until recurrence according to the VEGF rs699946 genetic variant using the patients who had been followed for 1 year or longer. During the 1-year follow-up period, there was no significant difference in the number of any of the treatments (intravitreal anti-VEGF drug, PDT, and combined therapy) among the three genotypes in either group ($P = 0.878$ and 0.477, respectively); contents of additional treatments (whether or not PDT was performed as additional therapy) did not differ among the two groups (Table 4). In the IVB patients who underwent one to three injections as the initial treatment, there was no significant difference in the number of injections used as the initial treatment among the three genotypes (AA 1.41, AG 1.57, GG 1.64, $P = 0.627$). There was also no significant difference in the frequency of recurrence ($P = 0.431$ and 0.342, respectively) or the period until it occurred ($P = 0.612$ and 0.980, respectively) among the three genotypes of either group (Table 5).

We examined the initial VA and that after treatment for all eyes in both the IVB group and the triple-therapy group based on the presence of rs699947. There was no significant difference in the mean baseline VA among the rs699947 AA, AC, and CC genotypes in either group ($P = 0.820$ and 0.691, respectively). Although the mean VA seemed to change notably in the rs699947 AA genotype whereas it did not change appreciably during the follow-up period in the other two genotypes (AC and CC) (Fig. 3), we found no relationship among these three genotypes and visual acuity in either group ($P = 0.257$ and 0.100, respectively). There was no difference in the frequency of AMD recurrence ($P = 0.645$ and 0.697, respectively) or the period until it was noted ($P = 0.987$ and 0.423, respectively) among the three rs699947 genotypes in either group.

Table 2 Distribution in the study population

Genotype, *n* (%)	Bevacizumab ($n = 94$)	Triple therapy ($n = 79$)
rs699946	$n = 91$	$n = 78$
AA	22 (24.2)	26 (33.3)
AG	47 (51.6)	44 (56.4)
GG	22 (24.2)	8 (10.3)
rs699947	$n = 92$	$n = 79$
AA	4 (4.3)	5 (6.3)
AC	33 (35.9)	37 (46.8)
CC	55 (59.8)	37 (46.8)
rs3025033	$n = 92$	$n = 79$
AA	65 (70.7)	63 (79.7)
AG	23 (25.0)	15 (19.0)
GG	4 (4.3)	1 (1.3)
rs3025035	$n = 92$	$n = 79$
CC	54 (58.7)	45 (57.0)
CT	31 (33.7)	31 (39.2)
TT	7 (7.6)	3 (3.8)

Discussion

In this study, VEGF rs699946 SNP was associated significantly with the visual prognosis of AMD after treatment with either IVB alone or after triple therapy. By evaluating the SNP before treatment, patients with neovascular AMD will be able to know what to expect after treatment, including what anti-VEGF drugs will be applicable. Recently, intensive studies have been performed on the association between various SNPs and the response to treatment of patients with AMD. Among the SNPs studied,

Table 3 Demographic characteristics by genotypes of VEGF rs699946

Group	Genotype	Mean age (years)	Female (%)	Right eye (%)	Mean follow-up (months)	Pretreatment VA	*P* value*
Bevacizmab	AA	74.9	9.1	54.5	14.2	0.659	0.101
	AG	74.8	36.2	46.8	12.6	0.493	
	GG	74.9	45.5	45.5	11.9	0.496	
Triple therapy	AA	75.4	23.1	61.5	12.9	0.684	0.4326
	AG	77.0	29.5	34.1	13.1	0.591	
	GG	75.2	12.5	62.5	14.1	0.628	

VA visual acuity

* The *P* values were generated by the ANOVA test

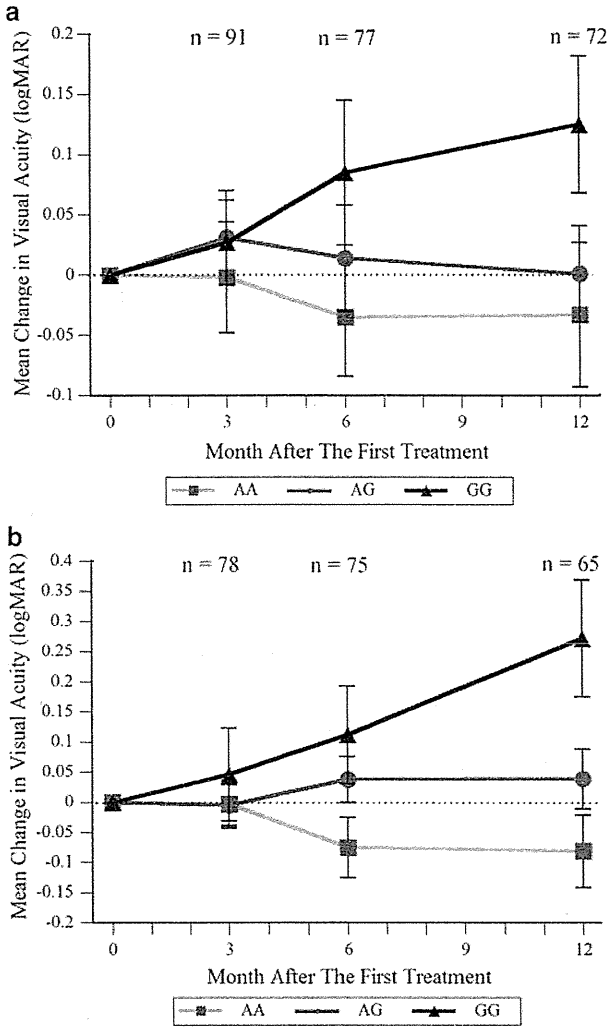

Fig. 2 Visual prognosis by VEGF rs699946 for the IVB and triple-therapy groups. The *two panels* show visual acuity (VA) over time in **a** the patients treated with intravitreal bevacizumab alone (IVB group) and, **b**, in the patients treated with photodynamic therapy with IVB and intravitreal triamcinolone acetonide (triple-therapy group). *Both graphs* show the mean VA changes ± SEM in logMAR from baseline among the three genotypes of VEGF rs699946 (*squares* AA, *circles* AG, and *triangles* GG). There was no difference in the mean baseline VA among the genotypes in either group. The G allele showed a significant trend toward better visual acuity in both the IVB group and in the triple-therapy group at 12 months ($P = 0.044$ and 0.010, respectively)

CFH Y402H was shown to correlate with the response to PDT, IVB, and ranibizumab [28–30]. These studies encourage us to personalize the treatment for each individual patient, with most suitable treatment being determined by studying the genotypes of each patient. However, the Y402H polymorphism is extremely rare in East Asia [31–36]; which markedly affects its applicability. Because the minor allele frequency of rs699946 was 43.9% in this study, this polymorphism is an attractive candidate for personalized treatment of AMD.

If the risk allele of an SNP is associated with VA prognosis, this risk allele frequency should have a trend. Because the risk-homo genotype has two risk alleles, it should have the worst prognosis, whereas the non-risk-homo genotype which contains no risk allele should have the best prognosis. Accordingly, this genotype, which contains one risk allele should have a "middle of the road" prognosis. Although CFH Y402H is a strong susceptibility gene polymorphism for AMD, the trend in the association between its risk allele and the response to treatment is not clear. Indeed, most reports show that the visual outcome is not always the best with the non-risk-homo genotype (TT) or the worst with the risk-homo genotype (CC). Goverdhan et al. [28] showed that those with a hetero genotype (TC) have the best VA changes after PDT for AMD, compared with those with the risk-homo genotype (CC) or the non-risk-homo genotype (TT). Similarly, Brantley et al. [30] reported that final visual acuity is better in those with the TC genotype than in those with the CC or TT genotypes. They reported that, when treated with IVB, AMD eyes with the TC genotype have a better visual prognosis than those with the CC genotype, and that the visual prognosis is almost the same for those with the TC and TT genotypes [29].

Unlike CFH Y402H, VEGF rs699946 SNP showed a clear trend in that, after treatment for AMD, visual prognosis was best with the GG genotype, medium with the GA genotype, and worst with the AA genotype. The frequency of the risk allele G would contribute to better prognosis. This association of the VEGF gene polymorphisms with visual prognosis in eyes with AMD seems to be inconsistent with previous studies that showed no association between AMD and VEGF gene polymorphisms [25, 37, 38]. At the same time, it does seem natural that the disease-susceptibility gene is different from the gene associated with the response to treatment. VEGF gene polymorphism may well contribute to the progress of AMD, rather than to the onset of AMD.

Although rs699946 has not been studied in depth, a recent report showed that the genotype of rs699946 was associated significantly with severe diabetic retinopathy [39]. In both diabetic retinopathy and AMD, it is well-known that VEGF contributes to pathologic neovascularization and to retinal edema. The association of rs699947, which, like rs699946, is on the promoter region, with the occurrence of diseases or with their prognosis has been investigated in various organs [24, 40–43]. The association of rs699947 and AMD has been denied in reports by Boekhoorn et al. [38] and by Lin et al. [10], whereas haplotype analysis did show an association of rs699947 with AMD [44]. Recently, Immonen et al. [25] showed that the frequency of rs699947 is significantly different in PDT nonresponders than in PDT responders. However, they did not evaluate its association to visual prognosis. In this

Table 4 Number and content of treatments during 1-year follow-up

Genotype	Number of treatments	*P* value*	Number of additional treatments with PDT	*P* value*
Intravitreal bevacizmab				
AA	2.52	0.878	0.05	0.658
AG	2.67		0.03	
GG	2.47		0.00	
Triple therapy				
AA	1.80	0.477	0.19	0.993
AG	1.64		0.18	
GG	2.17		0.17	

* The *P* values were generated by the ANOVA test

Table 5 Results from evaluation for recurrence

Genotype	Recurrence		*P* value (chi-squared)	Period until recurrence (days)	*P* value (ANOVA)
	+	−			
Intravitreal bevacizmab					
AA	9	12	0.431	171	0.612
AG	18	15		213	
GG	12	7		172	
Triple therapy					
AA	8	13	0.342	212	0.980
AG	21	17		213	
GG	2	4		194	

study, rs699947 did not affect visual prognosis after IVB or triple therapy for AMD but, because we had only four patients treated with IVB who had the AA genotype of rs699947 and five patients treated with triple therapy who had the AA genotype of rs699947, a larger cohort might show an association of rs699947 with visual prognosis. However, rs699946 would be the better genotype to be used clinically for personalized medicine because its distribution is more balanced than is that of rs699947.

The VEGF rs699946 is 5.8 kb upstream of the 5′ transcription start sites. The VEGF rs699946 captures and reflects one SNP on the promoter region (rs833060) and four SNPs on the VEGF gene (rs833068, rs833069, rs3024997, rs3025000) with high linkage disequilibrium. Because some of these SNPs correlate with VEGF production [45], a VEGF genotype might affect visual prognosis via the amount of VEGF produced. In addition to the amount of VEGF, the ratio of various VEGF isoforms can affect the prognosis of AMD because each VEGF isoform has its own role in both pathologic and physiologic neovascularization [46]. Considering that rs699946 exists upstream of the 5′ transcription start sites, it may affect splicing of VEGF and result in a changes in the expression level of VEGF isotypes such as $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$.

It might seem surprising that VEGF rs699946 SNP affected the visual outcome of eyes with AMD after two different treatments: IVB alone and triple therapy. Because the objective of IVB treatment is to eradicate CNV and to attenuate the exudative changes by suppressing VEGF, it seems plausible that variations in the VEGF gene change the visual outcome after treatment. However, the action of VEGF and IVB in triple therapy may be more complex. It was thought that IVTA and IVB suppress the VEGF upregulation that is induced by PDT [12]. Moreover, IVB may have a direct effect on eradication of CNV and on attenuation of the exudative change. Both of these mechanisms would lead to association of the VEGF gene variation with the visual outcome after triple therapy, but further studies are needed to clarify the biological functions of these VEGF genetic variants. However, because either anti-VEGF drugs alone or combined treatment that includes PDT with anti-VEGF drugs are currently the main treatment modalities for AMD, our findings of an association between VEGF gene polymorphism and the visual outcome after both IVB and triple therapy seem helpful.

In this study, VA in the IVB group tended to improve immediately after treatment whereas it tended to improve more gradually in the triple-therapy group. The Mont Blanc study shows that patients treated with IVB alone had better VA than patients treated with PDT + IVB. Furthermore, recent randomized clinical trials have shown that patients treated with IVB had a significantly better VA outcome than those treated with PDT + IVTA [47]. It can be

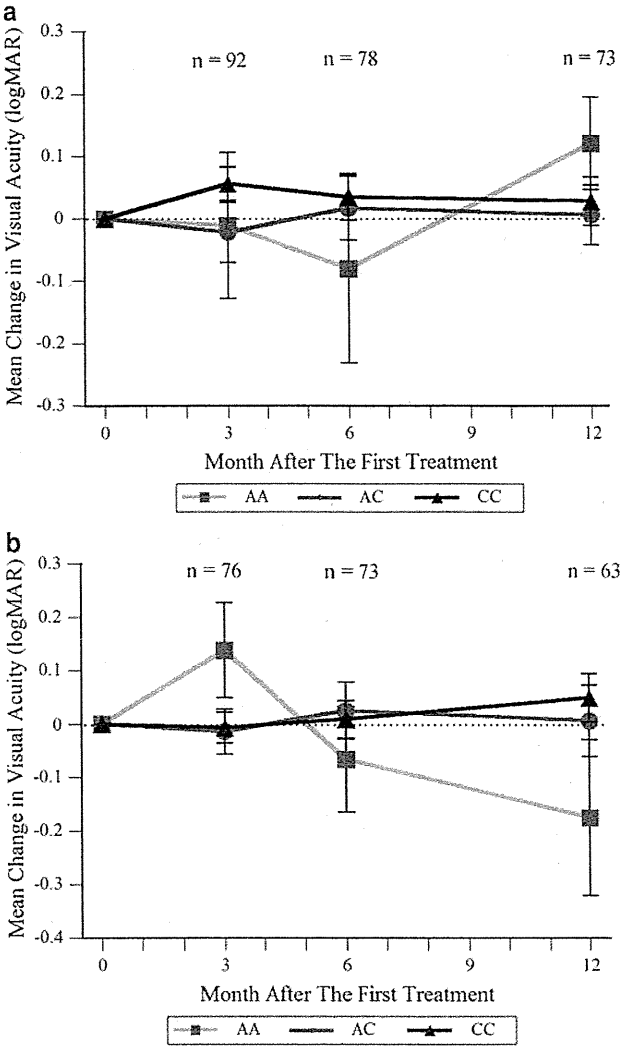

Fig. 3 Visual prognosis by VEGF rs699947 for the IVB and triple-therapy groups. **a** Visual acuity (VA) over time in the patients treated with intravitreal bevacizumab alone (IVB group) and, **b**, in the patients treated with photodynamic therapy with IVB and intravitreal triamcinolone acetonide (triple-therapy group). *Both graphs* show the mean VA changes ± SEM in logMAR from baseline among three genotypes of VEGF rs699947 rs699946 (*squares* AA, *circles* AC, and *triangles* CC). There was no difference in the mean baseline VA among the genotypes in either group. No relationship was seen among these three genotypes for visual acuity in either group ($P = 0.257$ and 0.100, respectively)

suggested that PDT and IVTA may delay the vision recovery even when we take into account the fact that the initial visual acuity was significantly different between the IVB group and the triple-therapy group in this study.

This retrospective study is limited by the size of the patient cohort and, although we selected tag SNPs with relatively high minor allele frequency, we could not evaluate the effects of some SNPs on visual prognosis. Because there were only a few patients with the GG genotype in rs3025033 and with the TT genotype in rs3025035 in the triple-therapy group, further investigation with a larger patient cohort may enable better evaluation of gene interactions and their relationship with treatment response. A further limitation of this study is the lack of evaluation of visual prognosis in the AMD patients without any treatment. Patients with the G allele in VEGF rs699946 might have better visual prognosis without treatment. In addition, the treatment method was not randomly assigned or chosen to follow solid criteria, which should be considered as a bias when interpreting our findings. Hidden baseline characteristics of the participants might affect the visual outcomes after treatment. The baseline VA of the patients with the AA genotype of rs699946 was slightly worse in both groups. Because the visual changes depended on the baseline VA, we cannot exclude the possibility that the differences of the baseline VA might affect the results of this study, even taking into account that they were not statistically significant. However, in this study, the rs699946 genotype was associated with the visual prognosis after both treatments. Further studies, for example a prospective study, would be needed to confirm the association between these variants and the visual prognosis of AMD.

Anti-VEGF drugs have become the principal treatment of neovascular AMD because the visual results are reported to be better than those obtained with PDT. However, numerous injections of the anti-VEGF drug may be required to maintain those visual benefits. To reduce the number of reinjections of anti-VEGF drugs, combination therapy, for example the triple therapy reported herein, has been evaluated. Because the rs699946 polymorphisms of the VEGF gene were associated with visual outcomes after both IVB and triple therapy, determination of the genotype of rs699946 can be of great help in predicting the visual outcome of AMD after treatment.

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Association between the SERPING1 Gene and Age-Related Macular Degeneration and Polypoidal Choroidal Vasculopathy in Japanese

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Abstract

Purpose: Recently, a complement component 1 inhibitor (*SERPING1*) gene polymorphism was identified as a novel risk factor for age-related macular degeneration (AMD) in Caucasians. We aimed to investigate whether variations in *SERPING1* are associated with typical AMD or with polypoidal choroidal vasculopathy (PCV) in a Japanese population.

Methods: We performed a case-control study in a group of Japanese patients with typical AMD (n = 401) or PCV (n = 510) and in 2 independent control groups—336 cataract patients without age-related maculopathy and 1,194 healthy Japanese individuals. Differences in the observed genotypic distribution between the case and control groups were tested using chi-square test for trend. Age and gender were adjusted using logistic regression analysis.

Results: We targeted rs2511989 as the haplotype-tagging single nucleotide polymorphism (SNP) for the *SERPING1* gene, which was reported to be associated with the risk of AMD in Caucasians. Although we compared the genotypic distributions of rs2511989 in typical AMD and PCV patients against 2 independent control groups (cataract patients and healthy Japanese individuals), *SERPING1* rs2511989 was not significantly associated with typical AMD (P = 0.932 and 0.513, respectively) or PCV (P = 0.505 and 0.141, respectively). After correction for age and gender differences based on a logistic regression model, the difference in genotypic distributions remained insignificant (P > 0.05). Our sample size had a statistical power of more than 90% to detect an association of a risk allele with an odds ratio reported in the original studies for rs2511989 for developing AMD.

Conclusions: In the present study, we could not replicate the reported association between *SERPING1* and either neovascular AMD or PCV in a Japanese population; thus, the results suggest that *SERPING1* does not play a significant role in the risk of developing AMD or PCV in Japanese.

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Introduction

Age-related macular degeneration (AMD) is the leading cause of visual loss in the developed world [1]. Several genes have been reported to be associated with this disease, including complement factor H [2–4] and the age-related maculopathy susceptibility 2/HtrA serine peptidase 1 (ARMS2/HTRA1) region [5,6], and subsequent studies have replicated the association between susceptibility genes and the development of AMD using a different ethnic cohort [7–10].

Inner choroidal vascular networks that terminate in polypoidal lesions are defined as polypoidal choroidal vasculopathy (PCV), and are typically visualized by indocyanine green angiography [11]. Whether PCV represents a subtype of neovascular AMD remains controversial; moreover, whether this condition represents inner choroidal vascular abnormalities or is a variety of choroidal neovascularization remains unknown [12]. Previous studies identified several genes that contribute to the development of PCV; however, almost all reported genetic risk factors for PCV are the same as for AMD [13–15], and this suggests that AMD and PCV share, at least in part, the same genetic background.

Studies in cohorts from both the United Kingdom and the United States have shown that the complement component 1 inhibitor (*SERPING1*) gene is positively associated with AMD [16]. However,

Table 1. Characteristics of the Study Population.

		Cases		Controls	
		tAMD	PCV	Control 1*	Control 2†
No. of participants		401	510	336	1194
Age	Mean ± SD	77.38±8.39	74.98±7.77	74.16±8.42	50.34±15.9
Gender	Men	287	372	142	493
	Women	114	138	194	701

tAMD, typical age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; SD, standard deviation.
*Cataract patients without age-related maculopathy.
†Healthy Japanese individuals.
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another study in a larger cohort (n = 7723 and 2327) which involved the same population could not replicate the finding of the previous study [17,18]. Recently, Lee et al. have shown that *SERPING1* is positively associated with AMD in Caucasians [19], but whether this gene is truly associated with AMD remains controversial.

Furthermore, the association of *SERPING1* with AMD has been evaluated also in Asians. Lu et al. examined the association in 194 AMD patients and 285 controls and reported that *SERPING1* is not associated with AMD in the Chinese population [20]. The association between PCV and *SERPING1* has also been evaluated in a smaller Chinese cohort (118 patients and 115 controls), also with negative findings [21]. So far, all Asian studies for *SERPING1* did use smaller cohorts than those of original studies and not consider their statistical power. For evaluating the true gene-disease association, it would be helpful to replicate the positive association reported in previous studies using a different ethnic cohort. The aim of this study, which involved a relatively large number of participants, was to investigate whether the *SERPING1* gene variants are associated with typical AMD or PCV in a Japanese population.

Materials and Methods

All procedures in this study adhered to the tenets of the Declaration of Helsinki. This study was approved by the Ethics Committee of each institute involved (Kyoto University Graduate School and Faculty of Medicine, Ethics Committee, the Ethical

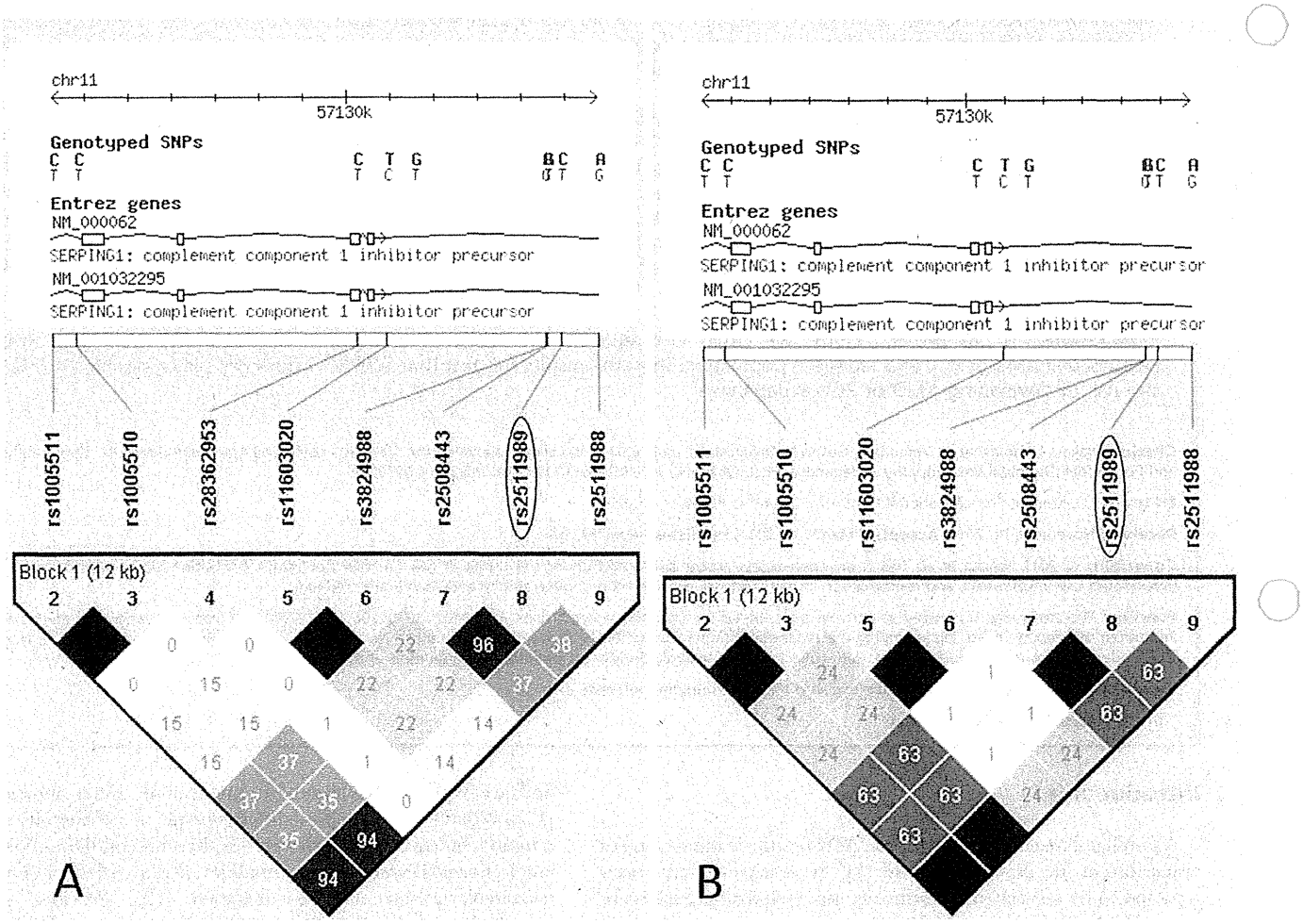

Figure 1. Linkage disequilibrium (LD) structure across the complement component 1 inhibitor (*SERPING1*) gene in Caucasian and Japanese populations. Genotype data were retrieved from HapMap CEU (Utah residents with ancestry from northern and western Europe; A) and JPT (Japanese in Tokyo, Japan; B) data sets. Haplotype blocks were determined using the "four-gamete rule" option in Haploview; all HapMap single nucleotide polymorphisms on *SERPING1* gene are in the same block in both populations. Each box provides estimated statistics of the coefficient of determination (r^2), with darker shades representing stronger LD.
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Committee of Fukushima Medical University, the Ethical Committee of Kobe City Medical Center General Hospital, the Ethical Committee of Ozaki Eye Hospital, the Ethical Committee of the Otsu Red Cross Hospital, the Ethical Committee of Nagahama City Hospital, and the Ethical Committee at Aichi Cancer Center). All of the patients were fully informed about the purpose and procedures of this study, and written consent was obtained from each.

In this study, 401 patients with typical AMD and 510 patients with PCV were recruited from the Department of Ophthalmology at Kyoto University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. The control group included 2 populations: (1) 336 individuals who underwent cataract surgery and had no age-related maculopathy (ARM) (Control 1) were recruited from the Department of Ophthalmology, Kyoto University Hospital, Ozaki Eye Hospital, Japanese Red Cross Otsu Hospital, and Nagahama City Hospital; and (2) 1194 healthy individuals who were recruited from the Aichi Cancer Center Research Institute as the general population control (Control 2). AMD and ARM were defined according to the International Classification System for ARM and AMD [22]. The diagnosis of PCV was based on indocyanine green angiography, which showed a branching vascular network that terminated in polypoidal swelling. Typical AMD were late AMD which showed classic choroidal neovascularization (CNV), occult CNV, or both. All diagnoses were made by 3 retina specialists (K.Y., A.T., and A.O.); a fourth specialist (N.Y.) was consulted when the subtype classification could not be decided on by the initial 3 reviewers. All of the subjects were unrelated and were of the Japanese descent.

Genomic DNAs were isolated from the peripheral blood of the subjects by using a DNA extraction kit (QuickGene-610L, Fujifilm, Minato, Tokyo, Japan). The samples of all the patients with typical AMD and PCV and of cataract patients were genotyped using a Taqman single nucleotide polymorphism (SNP) assay with the ABI PRISM 7700 system (Applied Biosystems, Foster City, CA). The individuals recruited from the Aichi Cancer Center Research Institute were genotyped using Illumina HumanHap 610 chips (Illumina Inc., San Diego, CA).

Linkage disequilibrium (LD) structures across the *SERPING1* gene were compared between the Caucasian and Japanese populations, using genotype data retrieved from the HapMap CEU and JPT data sets [23]. The retrieved data were loaded into Haploview to estimate LD parameters and to identify haplotype blocks [24]. Deviations in genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed using the HWE exact test. Statistical analyses for differences in the observed genotypic distribution were performed by the chi square test for trend; logistic regression analysis was performed for age and gender adjustments. The statistical power calculation was performed using QUANTO version 1.2 [25]. P values less than 0.05 were considered statistically significant.

Results

The demographic details of the study population are presented in Table 1. Because all SNPs of the *SERPING1* gene are in the same haplotype block, rs2511989 was selected as the haplotype-tagging SNP; rs2511989 was reported to be associated with the risk of AMD in previous studies [16,19] (Fig. 1). Details of allele and genotype counts and summary statistics for rs2511989 are shown in Table 2. The success rate of genotyping of rs2511989 was 98.1%, and the distributions of the genotypes for all study groups were in the Hardy–Weinberg equilibrium ($P>0.05$). Although we compared the genotype distributions of rs2511989 in typical AMD and PCV patients against 2 independent control groups (cataract patients without ARM and healthy Japanese individuals), *SERPING1* rs2511989 was not significantly associated with typical AMD ($P = 0.932$ and 0.513, respectively); furthermore, it was not significantly associated with PCV ($P = 0.505$ and 0.141, respectively). After correction for age and gender differences based on a logistic regression model, the difference in the genotype distributions remained insignificant ($P>0.05$). Table 3 shows the odds ratios adjusted for age and gender under various genetic models. We could not find a significant association in any genetic model.

Next, we calculated our statistical power to detect an association of a risk allele with the odds ratio reported in the previous study that investigated the association of rs2511989 with developing AMD. When we targeted the original study reported by Ennis (odds ratio 0.63) [16], our sample size had more than 90% power to detect the association (Table 2). In addition, the statistical power calculation revealed that our sample size could detect the gene-disease association for an odds ratio of 0.797 by more than 80%.

Discussion

In the present study, we investigated whether *SERPING1* gene variants are associated with typical AMD or with PCV in a Japanese population. We selected rs2511989 as the haplotype-tagging SNP, because this has been reported to be positively associated with the risk of AMD in Caucasians. The results of this study showed that *SERPING1* rs2511989 was not associated with the risk for typical AMD in a Japanese population; thus, the results did not support the hypothesis that an association between the *SERPING1* gene and AMD exists. Our sample size had more than 90% power to detect the association determined in the previous

Table 2. *SERPING1* rs2511989 Genotypic Distributions and Results of Association Tests and Power Analysis.

						vs Control 1			vs Control 2		
		GG	GA	AA	MAF	P Value	Adjusted P*	Power†	P Value	Adjusted P*	Power†
Cases	tAMD	293	102	6	0.142	0.932	0.687	93.6%	0.513	0.860	99.3%
	PCV	380	125	5	0.132	0.505	0.855	95.7%	0.141	0.678	99.2%
Controls	Control 1	248	76	10	0.144	-	-	-	-	-	-
	Control 2	859	308	27	0.152	-	-	-	-	-	-

tAMD, typical age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; MAF, minor allele frequency.
*Adjusted for age and gender.
†Statistical power for detecting the association reported in the previous study (odds ratio 0.63).
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Table 3. Odds Ratios in Various Genetic Models.

Group	Model	Adjusted Odds Ratio (95% Confidence Interval)*	
		vs tAMD	vs PCV
Control 1	Additive	0.938 (0.687–1.281)	0.972 (0.72–1.312)
	Dominant	1.283 (0.746–2.204)	0.598 (0.338–1.056)
	Recessive	0.934 (0.783–1.114)	1.283 (0.746–2.204)
Control 2	Additive	1.034 (0.716–1.491)	0.933 (0.673–1.294)
	Dominant	0.940 (0.470–1.879)	0.709 (0.349–1.440)
	Recessive	1.025 (0.839–1.254)	0.983 (0.823–1.174)

*Adjusted for age and gender.
tAMD, typical age-related macular degeneration; PCV, polypoidal choroidal vasculopathy.
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study in a Caucasian cohort (odds ratio 0.63) [16]. Furthermore, we found no evidence to support the role played by *SERPING1* rs2511989 in the susceptibility to PCV, and this finding is in agreement with that of the previous study in a Chinese population [21].

The reported association between AMD and *SERPING1* rs2511989 is shown in Table 4. The size of our Japanese cohort was similar to that of the original study [16]. Furthermore, the statistical power calculation revealed that our sample size could detect the gene-disease association for an odds ratio of 0.797 by more than 80%. Had there been a true protective effect of *SERPING1* gene variants for developing AMD at the same level as was reported in previous studies [16,19], the statistical power of our study would have detected such an association. Differences in the ethnicities of subjects might be 1 reason for the difference observed between the results of this study in a Japanese cohort and those of the previous study in a Caucasian cohort. Frequency of the minor allele of rs2511989 was reportedly greater in the earlier study in a Caucasian population than that of the present study in a Japanese population. In fact, in reference to the allele frequency data from the HapMap, all genetic variants across the *SERPING1* gene showed smaller minor allele frequency in Japanese than in Caucasians.

Another possible explanation for the differences between our findings and those of other studies in different ethnic cohorts may include a difference in the phenotypes of AMD. Numerous studies have reported that distinguishing features of Asian AMD include male predominance, unilateral presentation, comparatively low incidence of soft drusen, and greater prevalence of neovascular AMD and PCV [26–29]. To address these concerns, we classified AMD patients into those with typical AMD and those with PCV, but the possible hidden differences in the phenotypes cannot be excluded. Alternatively, considering the fact that genetic variants that are associated with a particular disease in 1 population may not necessarily be associated in another population [30–32]; moreover, it is possible that gene-disease association of *SERPING1* in populations from East Asia is very weak or absent as compared with Caucasian populations.

In this study, we used general population-based controls (Control 2). The possibility exists that some of the eyes in the control 2 group might have or develop AMD or PCV, and this might be a possible explanation for the negative results in this study. However, because the prevalence of AMD in the general population is estimated to be 0.5% in the Japanese population [33], the loss of the statistical power of association analysis must be negligible. In addition, we also performed a subset analysis on

Table 4. Comparison of Association Observed between AMD and *SERPING1* rs2511989.

Subject Group		Current Study (JP)			Mayo Subjects (US)		AREDS Subjects (US)		Ennis et al. (UK)		Ennis et al. (US)		Lee et al. (US)		Lu et al. (CH)	
Subjects		Case	Control 1	Control 2	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
No. of participants		401	336	1194	470	310	1221	295	479	479	248	252	556	256	194	285
Allele count	G	688	572	2026	569	363	1435	357	597	500	322	282	669	283	336	493
	A	114	96	362	371	257	1007	233	355	454	174	222	413	229	52	69
Genotype count	GG	293	248	859	179	103	436	115	191	132	100	79	213	74	147	215
	GA	102	76	308	211	157	563	127	215	236	122	124	273	135	42	63
	AA	6	10	27	80	50	222	53	70	109	26	49	70	47	5	3
MAF		0.142	0.144	0.152	0.395	0.415	0.412	0.395	0.373	0.475	0.351	0.441	0.382	0.447	0.134	0.123
P values		-	0.932	0.513	-	0.46	-	0.41	-	5.4×10^{-6}	-	0.0037	-	0.011	-	0.61

MAF, minor allele frequency.
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controls 2 with 55 years of age or older to minimize the possibility that some of the eyes in the control group might develop AMD or PCV. However, no new significant differences in the genotypic distributions were found in the current study (data not shown). Thus, we concluded that the result of the analysis using control 2 is valuable as reference data which supports a lack of association between *SERPING1* and both typical AMD and PCV in a Japanese population. Another limitation is about geographical difference of Control 1, which may influence genetic background of the participants. However, because the Japanese population has been reported to have a rather small genetic diversity, according to data from the SNP discovery project in Japan [34], geographical difference should not be affect our statistical results.

In conclusion, this study showed a lack of association between *SERPING1* and both typical AMD and PCV in a Japanese population; thus, the results suggest that *SERPING1* does not play a significant role in the risk of developing AMD or PCV in Japanese.

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Author Contributions

Conceived and designed the experiments: IN KY HN NY. Performed the experiments: IN NG HN HH. Analyzed the data: IN RY. Contributed reagents/materials/analysis tools: IN KY RY NG HN HH AT AO MS TI AO KM KT FM NY. Wrote the paper: IN KY RY.

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Association of Lesion Size and Visual Prognosis to Polypoidal Choroidal Vasculopathy

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• PURPOSE: To investigate the progression of vascular lesions of polypoidal choroidal vasculopathy (PCV) as viewed with indocyanine green angiography and the visual prognosis of these eyes.

• DESIGN: Retrospective case study.

• METHODS: We reviewed retrospectively the medical records of 88 consecutive patients (88 eyes) with PCV who had been examined with indocyanine green angiography for more than 2 years.

• RESULTS: Depending on the initial area of the vascular lesion, eyes were divided into smaller PCV (baseline area of lesion being < 1 disc area [DA], n = 22) and larger PCV (baseline area of lesion being ≥ 1 DA, n = 66). In larger PCV, the mean area of the lesion progressed significantly from 6.49 ± 8.96 mm^2 to 16.27 ± 14.19 mm^2 ($P < .0001$) with marked deterioration of visual acuity ($P < .0001$) during follow-up. In contrast, smaller PCV often showed minimal progression of the lesion, only limited exudative change, and the eyes maintained their initially good vision to the final visit. Smaller PCV lesions rarely progressed to extensive PCV lesions. Severe vision-threatening complications (ie, suprachoroidal hemorrhage, vitreous hemorrhage, and tears of the retinal pigment epithelium) were seen only in eyes with larger PCV, and in studying single nucleotide polymorphisms A69S of *ARMS2* genes, there was a significant difference in T allele frequency between individuals with smaller PCV and those with larger PCV (20.2% vs 79.8%; $P = .0235$).

• CONCLUSIONS: PCV with small vascular lesions shows minimal progression and no vision-threatening complications, and these eyes often maintain good visual acuity for a long time. (Am J Ophthalmol 2011;151: 961–972. © 2011 by Elsevier Inc. All rights reserved.)

POLYPOIDAL CHOROIDAL VASCULOPATHY (PCV) first was described as a new clinical entity with a unique form of choroidal vascular abnormality[1–3] and is characterized by a branching vascular network that terminates in polypoidal lesions seen by indocyanine green angiography.[4] Initially, vascular components of PCV are reported to be seen predominantly in a peripapillary location,[5] but macular PCV[6,7] and peripheral PCV[5,8] since have been reported. Yannuzzi and associates expanded the clinical spectrum of this disease and established the current understanding of PCV.[9] Today, macular PCV is more common in Asian populations and seems to be the condition most clinically significant.[7,10,11] To date, however, the pathogenesis of PCV is not understood fully, and it is still controversial whether it originates from an abnormality of the inner choroidal vessels or if it is a variant of choroidal neovascularization (CNV).[12]

PCV is accompanied often by recurrent serosanguineous detachments of the retinal pigment epithelium and neurosensory retina, and sometimes results in massive hemorrhagic complications with a sudden loss of vision.[13,14] Although the extent of visual disturbance in PCV varies, it generally is thought that the visual prognosis of PCV is better than that of exudative age-related macular degeneration (AMD).[11,12] In a previous report by Uyama and associates,[11] approximately half of the patients with PCV had a favorable visual outcome (better than 20/30) after being followed-up for more than 2 years. In PCV, other vision-threatening complications, such as type 2 CNV, disciform scar, or cystoid macular edema, are reported to be uncommon.[10,12]

Clinically, the size of the vascular lesions in PCV varies.[15] We sometimes see cases of PCV with a large lesion that show a poor response to the treatment and show progression of the lesion, resulting in poor visual prognosis. Tateiwa and associates reported that PCV with a large vascular network that extends beyond the vascular arcade is not uncommon, so we may speculate that vascular lesions of originally small PCV extend over time and result in these large PCV.[16] Clinically, however, we rarely see this type of progression.[17] PCV cases with a small lesion often show minimal exudative change and no progression of the lesion and can maintain good visual function for a long time.[18] Even with an exudative change, small PCV often show a favorable response to treatment.[19] Okubo and associates reported that a reddish-orange nodule alone, or that multiple reddish-orange nodules with a small subretinal hemorrhage, is a sign of a potentially

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TABLE 1. Characteristics of Patients with Polypoidal Choroidal Vasculopathy

	Total (n = 88)	Smaller Polypoidal Choroidal Vasculopathy (n = 22)	Larger Polypoidal Choroidal Vasculopathy (n = 66)	*P* Value
Gender (women/men)	28/60	8/14	20/46	.5971
Age (yrs)	70.4 ± 7.5	68.2 ± 6.9	70.8 ± 7.7	.3257
Hypertension	38	11	27	.4560
Smoking	14	4	10	.7365
Location of lesions (macular/peripapillary/peripheral)	79/8/1	22/0/0	57/8/1	.1881
Duration of symptoms (mos)	8.0 ± 11.9	7.0 ± 9.5	8.3 ± 12.6	.6484
Initial conditions				
Best-corrected visual acuity (logMAR)	0.37 ± 0.34	0.24 ± 0.29	0.42 ± 0.35	.0383
Area of lesion (mm^2)	7.75 ± 9.78	1.68 ± 0.53	9.79 ± 10.55	.0006
Greatest linear dimension (μm)	3412 ± 1647	1901 ± 464	3915 ± 1591	<.0001
Foveal thickness (μm)	403.5 ± 189.9	377.6 ± 175.4	412.2 ± 195.0	.4628
Thickness of neurosensory retina in the fovea (μm)	196.5 ± 83.8	209.1 ± 98.5	192.4 ± 78.6	.4204
Follow-up period (months)	46.4 ± 8.1	44.5 ± 6.7	47.0 ± 8.4	.2020
Treatment				
Photodynamic therapy	69	16	53	.3055
(Times of treatments)	1.9 ± 1.1	1.5 ± 0.7	2.1 ± 1.2	.0875
Anti-VEGF therapy	40	6	34	.0480
(Times of treatments)	2.9 ± 2.5	3.8 ± 3.7	2.7 ± 2.2	.3155
Pars plana vitrectomy	4	0	4	.2372
Cataract surgery	8	3	5	.2554
Final conditions				
Best-corrected visual acuity (logMAR)	0.62 ± 0.51	0.19 ± 0.33	0.76 ± 0.49	<.0001
Area of lesion (mm^2)	13.24 ± 13.47	4.13 ± 3.59	16.27 ± 14.19	.0002
Greatest linear dimension (μm)	4511 ± 2030	2761 ± 900	5095 ± 1967	<.0001
Foveal thickness (μm)	299.7 ± 189.5	235.3 ± 65.1	321.2 ± 211.7	.0651
Thickness of neurosensory retina in the fovea (μm)	197.1 ± 168.1	153.3 ± 38.4	211.7 ± 191.1	.1597
Changes during follow-up				
Best-corrected visual acuity (logMAR)	0.24 ± 0.51	−0.05 ± 0.36	0.34 ± 0.51	.0015
Area of lesion (mm^2)	5.48 ± 8.13	2.45 ± 3.53	6.49 ± 8.96	.0429
Greatest linear dimension (μm)	1100 ± 1204	860 ± 933	1180 ± 1278	.2838
Foveal thickness (μm)	−103.8 ± 221.9	−142.3 ± 163.6	−91.0 ± 237.8	.3500
Thickness of neurosensory retina in the fovea (μm)	0.6 ± 168.0	−55.8 ± 103.3	19.3 ± 181.4	.0691

logMAR = logarithm of the minimal angle of resolution; VEGF = vascular endothelial growth factor; yrs = years.

One disc area (DA) is estimated as 2.54 mm^2 on the basis of the 1 optic disc diameter of 1.8 mm. Based on the area of 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).

benign clinical course, so the clinical course of small and large PCV may be different.[18]

To study the progression of vascular lesions in PCV, it is essential to perform repeated indocyanine green angiography,[4] because most vascular lesions of PCV are located beneath the retinal pigment epithelium.[1–3] So far, however, there is little information on the long-term observation of the vascular components of PCV.[15] In the study described herein, we investigated progression of the vascular lesion of PCV using indocyanine green angiography and visual prognosis of affected eyes. Based on our findings, we report a new classification of PCV and the expected complications and visual prognosis of these 2 types of PCV.

METHODS

FOR THIS OBSERVATIONAL CASE STUDY, WE REVIEWED retrospectively the medical records of 88 consecutive patients (88 eyes) with symptomatic PCV who initially visited the Macula Service of the Department of Ophthalmology at Kyoto University Hospital between Jan-

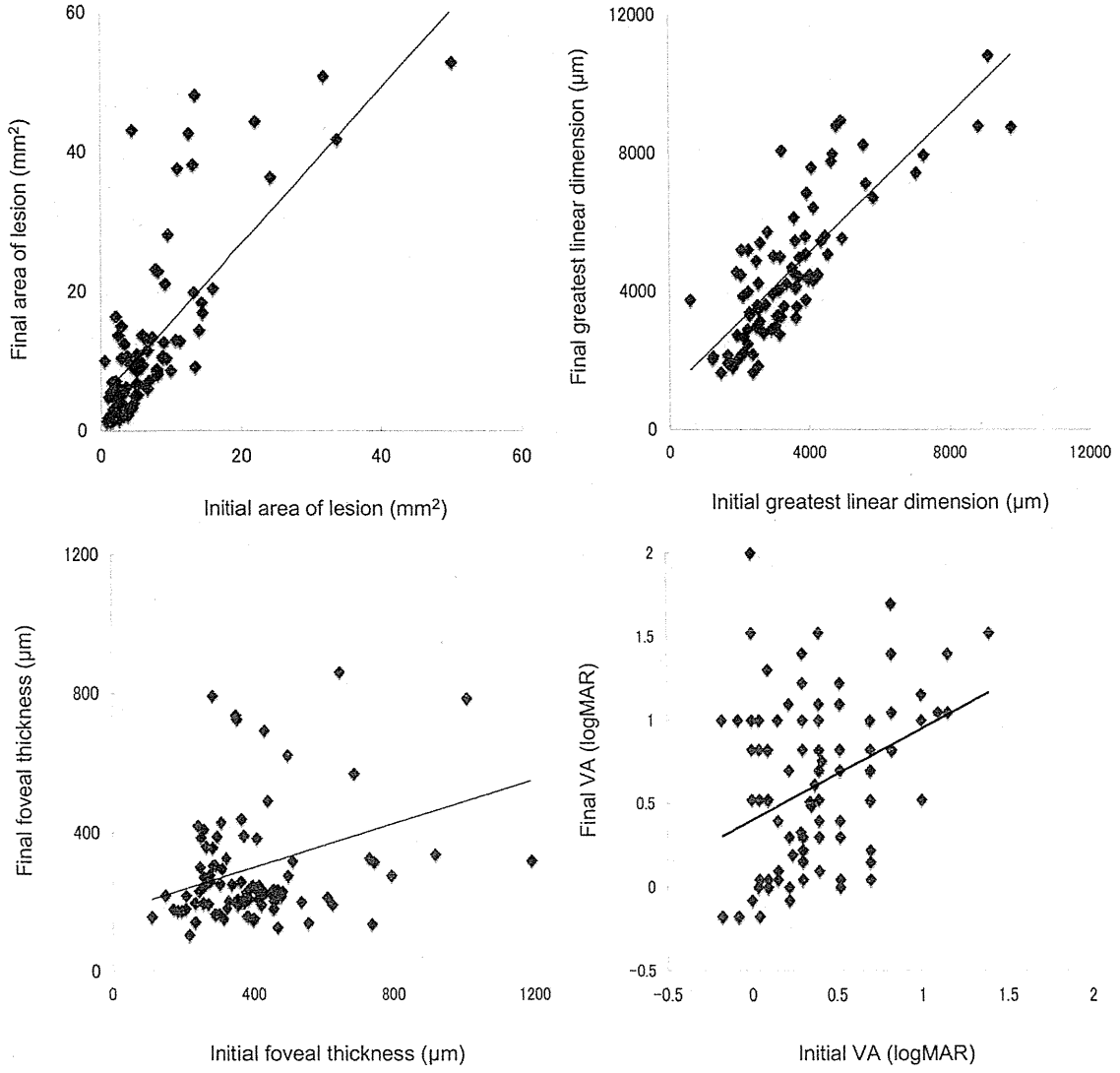

FIGURE 1. Scattergrams showing area of the lesion, greatest linear dimension, foveal thickness, and visual acuity (VA) in eyes with polypoidal choroidal vasculopathy (PCV) obtained at initial and final examinations. (Top left) Initial area is correlated significantly with final area of the lesion ($R = 0.801$; $P < .0001$). (Top right) Initial greatest linear dimension is correlated significantly with final greatest linear dimension ($R = 0.805$; $P < .0001$). (Bottom left) Correlations between initial and final foveal thickness ($R = 0.316$; $P = .0025$) and (Bottom right) initial and final VA ($R = 0.355$, $P = .0006$). VA measured with a Landolt chart was converted to logarithm of the minimal angle of resolution (logMAR) units.

uary 2004 and October 2007 and who had been examined with both fluorescein and indocyanine green angiography for more than 2 years after their initial visit. When both eyes were diagnosed as having PCV, 1 eye was selected randomly for inclusion in the current study.

The diagnosis of PCV was based on indocyanine green angiography, which shows a branching vascular network that terminates in polypoidal swelling. The polypoidal lesion can be a single polyp or a cluster of multiple polyps. In most cases, the reddish-orange nodule that had been seen by the ophthalmoscopic examination corresponded to the polypoidal lesion. Eyes with other macular abnormalities (ie, AMD, pathologic myopia, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, and other secondary CNV) were excluded from the current study. Eyes that were treated previously with focal laser photocoagulation, photodynamic therapy (PDT), vitrectomy, radiation therapy, or anti–vascular endothelial growth factor (VEGF) therapy also were excluded from the present study.

At the initial visit, all patients underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity (VA), determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, and optical coherence tomography (OCT). After fundus photographs were obtained, fluorescein and indocyanine green angiography

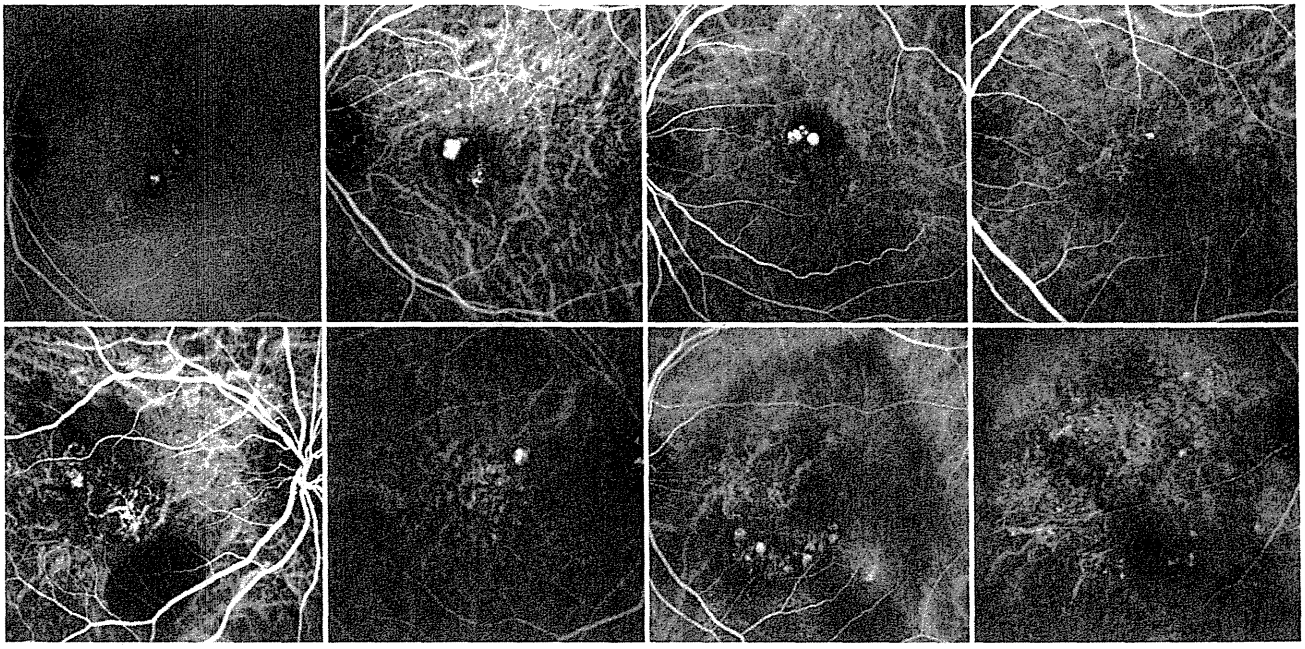

FIGURE 2. Indocyanine green angiograms obtained at the initial visit from eyes with polypoidal choroidal vasculopathy (PCV). All eyes showed the branching vascular network that terminated in a polypoidal lesion, although the lesions varied in size. (Top) Indocyanine green angiograms in the group with smaller PCV. (Bottom) Indocyanine green angiograms in the group with larger PCV.

were performed on each patient using a confocal laser scanning system (HRA-2; Heidelberg Engineering, Dossenheim, Germany). In all patients, VA measurement and OCT examination were performed at each follow-up visit. At follow-up visits, angiography was performed if necessary, although all patients in the current study were examined with angiography several times during follow-up. In the study described herein, the angiograms obtained at the initial visit were compared with the final angiograms.

In the current study, greatest linear dimension and area of the lesion were determined based on the indocyanine green angiography, using the software built into the HRA-2 machine. Greatest linear dimension included the entire PCV vascular lesion, including polypoidal lesion, branching vascular network vessels, and any type 2 CNV. The area of the vascular lesion was measured manually with the software that came with the HRA-2. The pigment epithelial detachment, without underlying vascular components, was not included in measurement of the greatest linear dimension and area of the lesion. In the current study, 1 optic disc area (DA) is equal to 2.54 mm^2, on the basis of 1 optic disc diameter being equal to 1.8 mm. Based on the area of the lesion at the initial visit, we classified the eyes into either the smaller PCV group (baseline area of lesion $<$ 1 DA) or the larger PCV group (baseline area of lesion $\geq$ 1 DA) to compare the clinical course of the 2 groups.

We also compared the initial OCT measurement and VA with values obtained at the final visit. To compare the difference in VA, VA measured with a Landolt chart was converted to logarithm of the minimal angle of resolution units. Using OCT images, we obtained 2 measurements (foveal thickness and thickness of the neurosensory retina in the fovea) with a caliper that was built into the software of the OCT machine. Foveal thickness was defined as the distance between the vitreoretinal interface and the retinal pigment epithelium; thickness of the neurosensory retina

showing a small protrusion of the retinal pigment epithelium corresponding to the PED. (Second row left) Fluorescein angiogram obtained at the initial visit showing occult choroidal neovascularization corresponding to a branching vascular network. (Second row right) Indocyanine green angiogram revealing large vascular components of PCV consisting of a polypoidal lesion (arrow) and a branching vascular network (long arrow). The area of the PCV lesion was 5.87 mm^2. (Third row left) Despite 3 anti–vascular endothelial growth factor treatments, the vascular lesion progressed. Fundus photograph obtained at 33 months after the initial visit showing a large serosanguineous PED. (Third row right) Sectional image obtained by OCT (with the arrow shown in the fundus photograph) showing a steep protrusion of retinal pigment epithelium, which is reflective of the large PED. (Bottom left) Fluorescein angiogram showing occult choroidal neovascularization corresponding to the branching vascular network. (Bottom right) Indocyanine green angiogram showing progression of the polypoidal lesions and extension of the branching vascular network. The area of the PCV lesion increased to 9.40 mm^2.