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 $\geq$ 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 $\leq$1800 μm in the first group, 1800 to 5400 μm in the second group, and $\geq$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

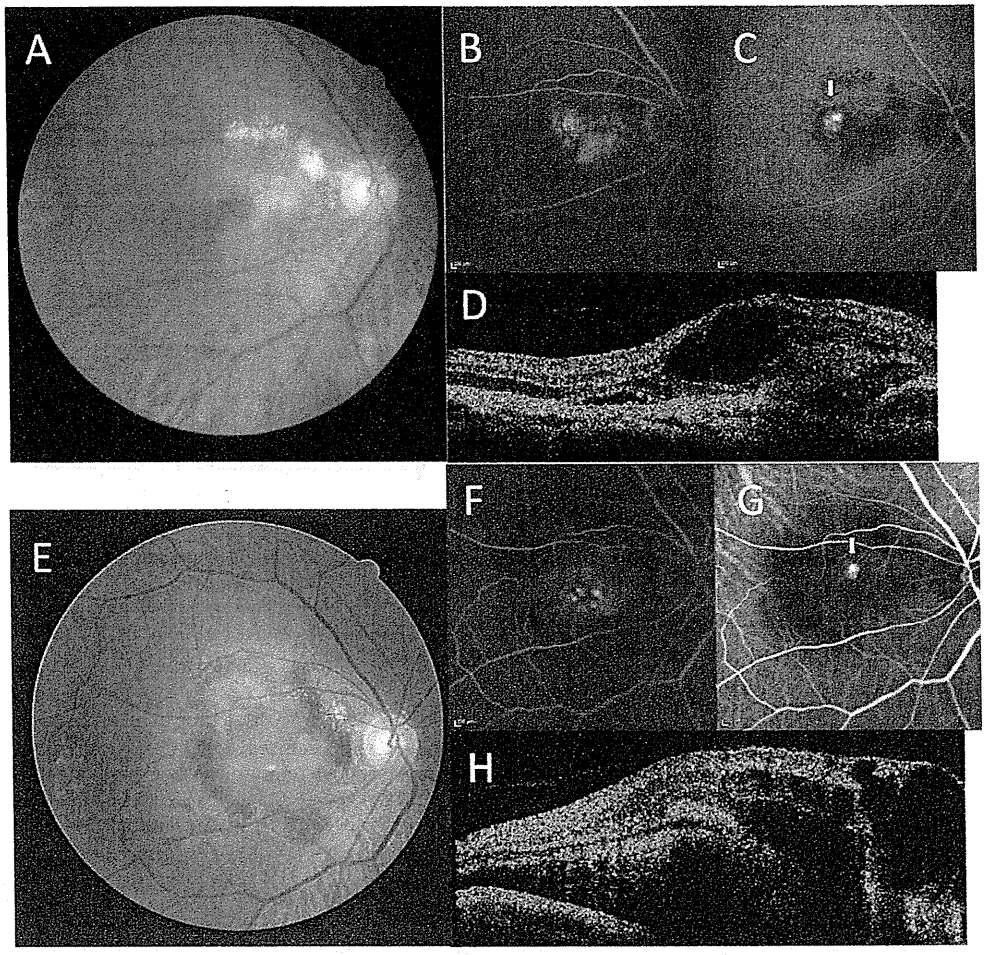

Figure 1. Fundus photographs (**A, E**), fluorescein angiographs (**B, F**), indocyanine green angiographs (**C, G**), and optical coherence tomographs (**D, H**) of a patient who received additional treatment within 3 months after the first PDT. This 72-year-old man with PCV in his right eye underwent 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

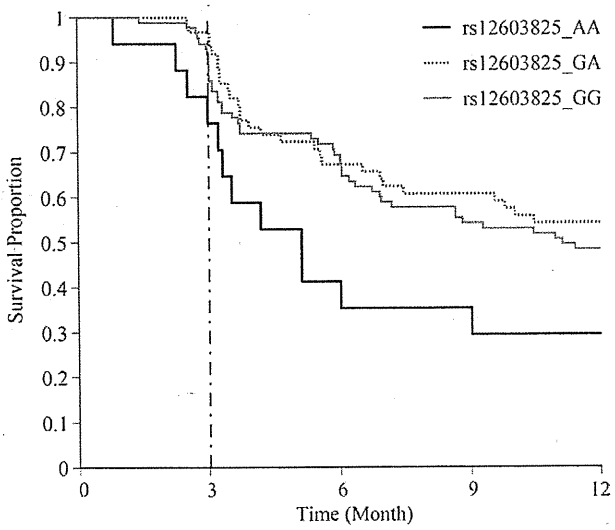

Figure 2. Overall survival analysis curve for the retreatment-free period among patients with the genotype of rs12603825. Patients with AA genotype were administered additional treatment after the first PDT within a significantly shorter period of time than those with other genotypes (P = 0.0038).

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

						Screening Sample (n = 31)	All Sample (n = 167)		
SNP	Chr*	Position*	Ref.†	Var.†	Gene*	*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 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

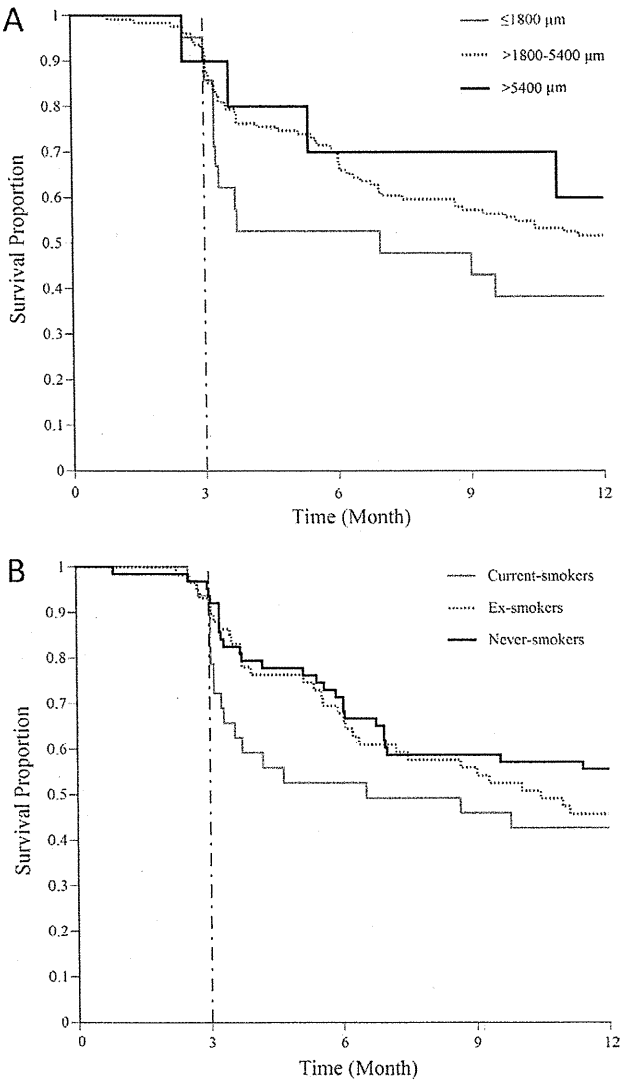

Figure 3. Overall survival analysis curve for the retreatment-free period by the 3 groups of GLD (**A**) and smoking status (**B**). There was no significant difference among these groups (P = 0.214 and 0.166, respectively), although borderline evidence of an association was observed between those who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

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.

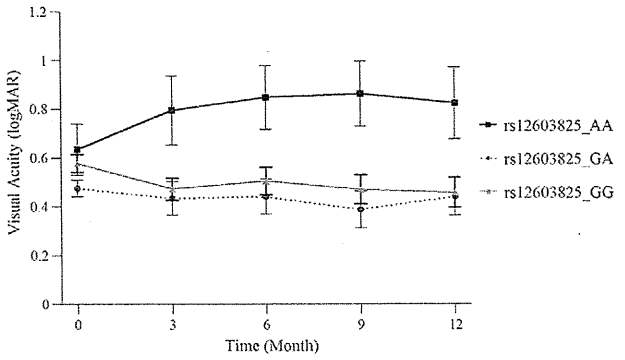

Figure 6. Visual prognosis by SERPINF1 rs12603825 after PDT. Visual outcomes of patients with the AA genotype were significantly worse than those with other genotypes (P = 0.013). Error bars represent ± 1 standard error of the mean. logMAR = logarithm of the minimum angle of resolution.

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.

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Footnotes and Financial Disclosures

Originally received: August 13, 2010.
Final revision: November 10, 2010.
Accepted: December 10, 2010.
Available online: March 24, 2011. Manuscript no. 2010-1115.

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Presented at: The American Academy of Ophthalmology Annual Meeting, October 16–19, 2010, Chicago, Illinos.

Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Supported in part by grants-in-aid for scientific research (Nos. 21249084 and 200791294) from the Japan Society for the Promotion of Science, Tokyo, Japan, and the Japan National Society for the Prevention of Blindness, Tokyo, Japan. The funding organizations had no role in the design or conduct of this research.

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ORIGINAL RESEARCH

Comparison of the effect between pegaptanib and ranibizumab on exudative age-related macular degeneration with small lesion size

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Purpose: To compare the effect of pegaptanib versus ranibizumab on exudative age-related macular degeneration (AMD) with small lesion size.

Methods: This is a retrospective study of 81 eyes from 78 patients with exudative AMD treated and followed up over 12 months. Patients with baseline best corrected visual acuity (BCVA) under 20/400 and with a greatest linear dimension of lesion over 4500 μm were excluded from the study. Twenty-six eyes from 25 patients were treated with three consecutive intravitreal injections of pegaptanib (IVP group) and 55 eyes from 54 patients were treated with three consecutive ranibizumab injections (IVR group). Each therapy was repeated as needed. The alteration in BCVA was evaluated in the IVP and IVR groups.

Results: No differences were detected in baseline parameters between the IVP and IVR groups. The mean BCVA (logMAR) at month 1, 3, 6 and 12 after the initial treatment was improved from baseline in the IVP group (−0.095, −0.17, −0.18 and −0.18, respectively) and in the IVR group (−0.077, −0.15, −0.17 and −0.11, respectively), which was statistically significant. There was no difference in the change in mean BCVA between IVP and IVR groups at the same time periods.

Conclusions: The visual outcome of IVP was equivalent with IVR in exudative AMD with small lesion size.

Keywords: pegaptanib, ranibizumab, age-related macular degeneration, small lesion size

Introduction

Intravitreal injection of anti-vascular endothelial growth factor (VEGF) agent is currently the main treatment for subfoveal choroidal neovascularization (CNV) due to age-related macular degeneration (AMD), a leading cause of central visual loss in the elderly in industrialized countries.[1,2] Currently, there are two anti-VEGF agents approved to treat exudative (or neovascular) AMD; pegaptanib sodium, a specific anti-$VEGF_{165}$ aptamer and ranibizumab, a nonselective anti-VEGF-A antibody. Previous randomized control studies demonstrated a significant improvement in the mean visual acuity of exudative AMD patients treated with intravitreal injection of ranibizumab (IVR),[3–5] while those treated with intravitreal injection of pegaptanib (IVP) showed no improvement in the mean visual acuity.[6] However, recent reports documented that visual loss after 24 months of monthly IVR or at 24 months after IVR with a pro re nata (as needed) regimen was associated with abnormalities of retinal pigment epithelium (RPE), excessive subretinal fibrosis, and atrophic scar.[7,8] We hypothesized that those results might be attributable to nonspecific suppression of VEGF, a potent survival factor for photoreceptor cells,[9] choroidal vascular endothelial cells,[10] and RPE[11,12]

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http://dx.doi.org/10.2147/OPTH.S30310

thus the subtype-specific anti-VEGF therapy should be selected as the main intervention to treat exudative AMD. To our knowledge, no study has been published to compare the effectiveness between IVP and IVR for exudative AMD with respect to lesion size.

In this study, we performed a comparative assessment to determine whether the visual outcomes of IVP and IVR were different in exudative AMD with relatively smaller lesion size and better baseline visual acuity.

Subjects and methods

The records of 185 consecutive exudative AMD patients treated by IVP or IVR and followed up over 12 months were retrospectively reviewed. All patients received detailed ophthalmic examinations, including best corrected visual acuity (BCVA) measurements, slit lamp biomicroscopy of their fundi, color fundus photography, fluorescein angiography (FA), indocyanine green angiography (ICG) and optical coherence tomography (OCT). Patients with baseline BCVA under 20/400, those with a greatest linear dimension (GLD) of lesion over 4500 μm, and patients who had received previous therapy for AMD were excluded from the study. Patients with past histories of retinal vessel occlusion, uveitis, rhegmatogenous retinal detachment or glaucoma were also excluded. Following these protocols, 81 eyes of 78 patients were included for analysis.

From October 2008 to March 2009, all patients were treated by IVP. After ranibizumab became available in Japan (April 2009), IVR was selected as the main intervention and IVP was used for patients with a risk of brain infarction. In the IVP group (26 eyes of 25 patients), all patients received three consecutive IVP injections at 6 weekly intervals as the initial treatment. In the IVR group (55 eyes of 53 patients), all patients received three consecutive monthly IVRs for the initial treatment as previously described. Patients were then followed up with monthly examinations of the lesions[13,14] and additional IVP or IVR was performed as needed, namely when sustained or recurrent serous retinal detachment, macular edema or hemorrhage was recognized by fundoscopy or OCT. Two patients in the IVP group received IVR 6 months after the initial IVP since the physician considered that IVP was not effective enough to reduce CNV lesions (including serous retinal detachment and macular edema). For those patients, we excluded the data for BCVA at 12 months after the initial treatment from the analysis. However, we counted each IVR as one treatment in the analysis with respect to the number of treatments.

For statistical analysis, we first compared gender, age, BCVA, GLD at baseline between the IVP and IVR groups. Changes in BCVA were then compared until 12 months after the initial treatment. Visual acuities were determined using a Landolt C chart, and were converted to logarithm of the minimum angle of resolution (logMAR) values for calculation. An F-test for homoscedasticity of variance followed by a two-tailed *t*-test or a chi-square test was performed to compare any two groups. *P* values of 0.05 or less were considered to be statistically significant.

Results

The data summary of AMD patients treated by IVP or IVR is shown in Table 1. No baseline parameter showed significant difference between the IVP and IVR groups. The F-test indicated homoscedasticity of variance in BCVA between the IVP and IVR groups (F-value = 0.49, $P = 0.49$). In the time course analysis, the mean BCVA was significantly improved compared with the baseline BCVA in each group (Figure 1). Although the IVR group showed a decrease in the mean BCVA at the 12 month follow-up, there was no significant difference between the IVP group and the IVR group at any time period measured. For BCVA measurements, about 25%–30% of patients gained more than 0.3 LogMAR during 12 months after the initial therapy, whereas about 10% of patients lost more than 0.3 LogMAR during the same time period in both groups (Figure 2). There was no significant difference in the proportion of BCVA change in the IVP

Table 1 Data summary of the participants treated by intravitreal injection of pegaptanib or ranibizumab

	IVP (n = 26)	IVR (n = 55)	*P* value
Male/female	19/6	35/18	0.37†
Age (years)	72.2 ± 11.0	74.3 ± 9.7	0.40*
Age range (years)	50–89	51–92	
Lesion type (eyes)			
Predominantly classic	6	8	0.65†
Minimally classic	6	11	
Occult with no classic	4	14	
With PCV	10	22	
Baseline BCVA (LogMAR)	0.44 ± 0.37	0.50 ± 0.36	0.49*
BCVA range	20/400–20/20	20/400–20/20	
Baseline GLD (μm)	2337 ± 1014	2825 ± 912	0.10*
GLD range (μm)	686–4290	810–4232	
Number of injections/year	4.6 ± 2.2	5.1 ± 2.3	0.39*
Number of injections/year range	3–9	3–11	

Notes: Values are presented as mean ± SD when applicable. *Unpaired *t*-test; †chi-square test.

Abbreviations: IVP, intravitreal injection of pegaptanib; IVR, intravitreal injection of ranibizumab; BCVA, best corrected visual acuity; GLD, greatest linear dimension; PCV, polyploidal choroidal vasculopathy.

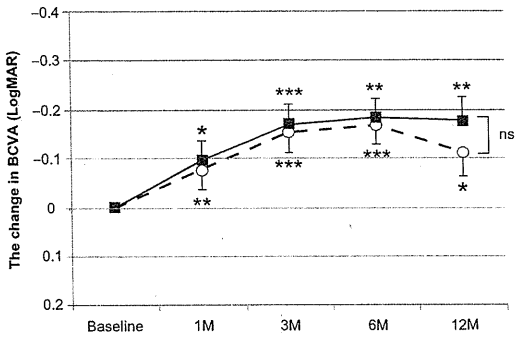

Figure 1 Changes in the best corrected visual acuity (BCVA) after intravitreal pegaptanib or ranibizumab.
Notes: Squares with solid lines: pegaptanib; Circles with dashed lines: ranibizumab. Values represent means ± standard error in the mean. *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$ compared to baseline.
Abbreviation: ns, not significant.

group versus the IVR group ($P = 0.68$). An accumulation of subfoveal hard exudates was found in one case in the IVP group, whereas four cases showed atrophic scars and three cases showed subfoveal fibrosis in the IVR group, and those were associated with a deterioration of BCVA 12 months after the initial treatment.

Discussion

We compared the effect of IVP versus IVR on exudative AMD with relatively small lesion size, and demonstrated that the visual outcome was not significantly different between the IVP and IVR groups. In other words, IVP was a good modality of choice for exudative AMD without severe visual disturbance and with smaller GLD at baseline.

Currently, anti-VEGF therapies are the leading modalities for exudative AMD.[15–17] Many reports demonstrated that IVR remarkably attenuated the activity of CNV and improved the average visual outcome. However, recent reports have shown

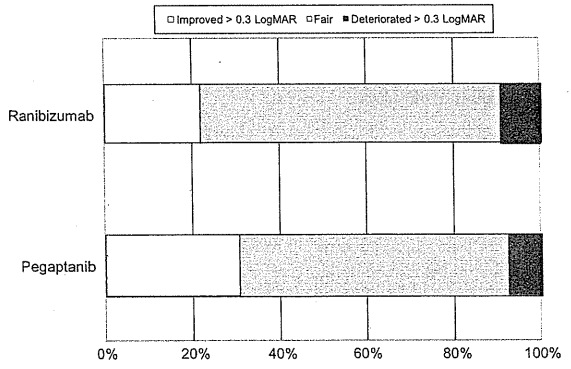

Figure 2 Proportion of the change in the BCVA (LogMAR) between baseline and after 12 months of intravitreal pegaptanib or ranibizumab in the exudative AMD patients.

that secondary visual loss, occurring at or after month 24 of IVR, was associated with abnormalities of the retinal pigment epithelium (RPE), subretinal fibrosis and atrophic scar,[7,8] which suggested the risk of nonspecific suppression of VEGF by ranibizumab. Efforts were made to decrease the number of IVR injections to treat exudative AMD,[5,13,14] but the use of IVP may be considered as an alternative therapy for exudative AMD with small lesion size.[18] $VEGF_{165}$ is known as the major inducer of abnormal blood vessel growth and leakage in wet AMD,[19,20] but all VEGF-A isoforms are key angiogenic and neuroprotective factors for several tissues.[9–12,21–23] Nonspecific inhibition of all VEGF-A isoforms might reduce the ability to tolerate several kind of stresses in the photoreceptor, RPE and normal choroidal endothelial cells. The abnormalities of RPE and atrophic scars found in the cases treated with monthly IVR might reflect the lack of VEGF-mediated neuroprotection for the cells. Interestingly, we found that four cases showed atrophic scars and three cases showed subfoveal fibrosis in the IVR group while these findings were not observed in the IVP group in the present study. To avoid the risk of oversuppression of physiological VEGF effects, many studies have been conducted to reduce the number of IVR injections.[5,13,14] A recent prospective study has demonstrated good visual outcomes of exudative AMD patients by using IVP as a maintenance therapy after IVR.[24] Other studies reported that good visual stability was obtained with IVP monotherapy in selective cases, particularly those in the early stage.[25,26] Since the pathogenesis of CNV is thought to be associated with $VEGF_{165}$ and $VEGF_{121}$,[27,28] IVP monotherapy may not be sufficient to suppress all CNV. However, our results have demonstrated that IVP could be a useful modality of choice for the patients with exudative AMD having small lesion size.

The major limitation of the present study was the nonrandomized and retrospective nature of the study and the relatively small number of subjects. Hence, it is important to evaluate the results of randomized control trials for IVP and IVR with a large number of subjects to determine the comparative effectiveness of these therapies, particularly for exudative AMD with small lesion size. Further investigations will be needed to determine the correct indications for use of IVP and IVR for exudative AMD.

In conclusion, IVP may be an effective therapy for BCVA over a 12 month period in patients with exudative AMD and lesions less than 4500 μm in size.

Acknowledgments

This study was supported by Grant-in-Aid (C) 23592567 from the Ministry of Education, Science, and Culture,

Tokyo, Japan (S.H.), and by a grant from the Takeda Science Foundation (S.H.). The funding organizations had no role in the design or conduct of this research.

Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Effect of photodynamic therapy (PDT), posterior subtenon injection of triamcinolone acetonide with PDT, and intravitreal injection of ranibizumab with PDT for retinal angiomatous proliferation

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Background: The purpose of this work was to compare the efficacy of photodynamic therapy (PDT) with or without posterior subtenon injections of triamcinolone acetonide (STA) or intravitreal injections of ranibizumab (IVR) for retinal angiomatous proliferation (RAP).

Methods: Thirty-seven eyes from 33 consecutive patients with RAP were treated by PDT monotherapy (Group 1), PDT combined with STA (Group 2), or PDT combined with IVR (Group 3). The best-corrected visual acuity, greatest linear dimension, central retinal thickness, and number of treatments were compared among the three groups.

Results: The change in mean best-corrected visual acuity (logMAR) at month 3, 6, and 12 after the initial treatment was better in Group 2 (−0.13, −0.23, and −0.21, respectively) and Group 3 (−0.018, 0.0028, and −0.0067, respectively) than in Group 1 (0.13, 0.19, and 0.23, respectively); Group 1 versus Group 2 was statistically significant ($P = 0.018$). The mean central retinal thickness was reduced from baseline in all groups, but the reduction amplitude was significantly greater in Group 2 than in Group 1 and Group 3. The mean number of treatments was significantly lower in Group 2 (1.1 ± 0.4) and Group 3 (1.5 ± 0.5) than in Group 1 (2.9 ± 0.9) in the 12 months after the initial treatment.

Conclusion: Treatment with STA + PDT may be an effective therapy for RAP lesions over 12 months of follow-up.

Keywords: retinal angiomatous proliferation, photodynamic therapy, triamcinolone acetonide, ranibizumab, combined therapy

Introduction

Age-related macular degeneration (AMD) is a leading cause of central vision loss in the elderly in industrialized countries.[1] The number of patients with AMD has increased remarkably over the years, and a further increase in patients with severe visual impairment due to AMD is anticipated.[2] Advanced AMD is clinically classified into atrophic AMD and exudative AMD. Exudative AMD is further classified into typical neovascular AMD, polypoidal choroidal vasculopathy, and retinal angiomatous proliferation (RAP).[1] These phenotypes are known to have different characteristics in their natural courses and their responses to interventions, such as photodynamic therapy (PDT) and antivascular endothelial growth factor (VEGF) therapy, although the reasons are unknown.[3–5] Several studies have reported a poor response to PDT monotherapy in patients with RAP lesions.[6,7] Hence, it is common to perform PDT combined with intravitreal injections of triamcinolone acetonide (IVT) or anti-VEGF

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http://dx.doi.org/10.2147/OPTH.S29718

therapy for RAP.[8,9] Several studies were conducted to address the comparative effectiveness between PDT monotherapy and the combination of PDT with IVT or anti-VEGF therapy against RAP lesions, but their conclusions were not consistent.[10,11] Rouvas et al reported that IVT + PDT was more effective than intravitreal ranibizumab (IVR) + PDT for RAP lesions.[10] In contrast, Saito et al reported that intravitreal bevacizumab + PDT was likely to be more effective than IVT + PDT in a Japanese RAP cohort.[11] This discrepancy might reflect the different anti-VEGF agents used in the two studies, so more replication studies are needed.

A posterior subtenon injection of triamcinolone acetonide (STA) is an alternative method to deliver triamcinolone acetonide to the posterior retina. Although IVT may cause an elevation in intraocular pressure and cataracts as complications,[12,13] STA might have fewer side effects in terms of inducing intraocular pressure elevation or cataracts than IVT because STA should act on the retina transsclerally and thus affect the lens and trabecular meshwork less than IVT.[14,15] However, to our knowledge, only a few published studies have compared the effectiveness of PDT combined with STA.[13] Therefore, in this study, we performed a comparative assessment between STA + PDT, IVR + PDT, and PDT alone in RAP patients.

Subjects and methods

This study was approved by the institutional review board of the Kobe University Graduate School of Medicine, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. All cases in this study were Japanese individuals recruited from the Department of Ophthalmology at Kobe University Hospital and Hyogo Prefectural Amagasaki Hospital in Japan.

This was a retrospective study of 37 eyes from 33 consecutive patients with RAP treated and followed up for more than 6 months. All patients received detailed ophthalmic examinations, including best-corrected visual acuity (BCVA) measurements, slit lamp biomicroscopy of their fundi, color fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Visual acuities were determined using a Landolt C chart, and were converted to a logarithm of the minimum angle of resolution (logMAR) values for calculation. The diagnosis and staging of RAP was performed as previously described.[16] Patients with past histories of retinal vessel occlusion, uveitis, rhegmatogenous retinal detachment, or glaucoma were excluded.

Fourteen eyes from 12 consecutive patients recruited by May 2006 were treated by PDT monotherapy (Group 1), 12 eyes from 10 consecutive patients recruited from June 2006 to March 2009 were treated by PDT combined with STA (Group 2), and 11 eyes from 11 consecutive patients recruited thereafter were treated by PDT combined with IVR (Group 3). No patients in this study received previous therapy except for one patient in Group 2 who underwent PDT monotherapy 4 months earlier. For STA + PDT, a small incision was made in the lower temporal conjunctiva, and 20 mg of triamcinolone acetonide was injected retrobulbarly with a blunt needle 4–7 days before PDT. For IVR + PDT, 0.3 mg of ranibizumab was injected intravitreally with a 30-gauge needle 3–7 days before PDT. Patients were examined 3, 6, 9, and 12 months after the initial treatment, and were retreated if persistence or recurrence of intraretinal, subretinal, or subretinal pigment epithelium fluid, or any increase in retinal thickness was found by funduscopy or optical coherence tomography. The retreatment was done according to the same protocol as for the initial treatment.

For statistical analysis, we compared the gender, age, BCVA, greatest linear dimension, and central retinal thickness at baseline among the three groups. Changes in BCVA and central retinal thickness were then compared every 3 months until month 12 after the initial treatment. The number of treatments performed during the first 12 months after the initial treatment was compared among the groups. To evaluate the influence of STA on intraocular pressure, intraocular pressure values before and 2 weeks after STA were measured for the STA + PDT group. The Wilcoxon signed-rank test was performed to compare any two time points within the group and an analysis of variance was used to make a comparison between the groups. P values of 0.05 or less were considered to be statistically significant. StatView-J software (v 5.0; Abacus Corporation, Baltimore, MD) was used for statistical analyses.

Results

A summary of the data for the RAP patients is shown in Table 1. No differences were detected in baseline parameters between the three groups. The transition of values in mean BCVA (logMAR) and mean central retinal thickness are shown in Table 2. The change in mean BCVA at months 3, 6, and 12 after initial treatment was better in Group 2 (–0.13, –0.23, and –0.21) and Group 3 (–0.018, 0.0028, and –0.0067) than in Group 1 (0.13, 0.19, and 0.23); Group 1 versus Group 2 was statistically significant ($P = 0.018$, Figure 1). The mean BCVA was significantly better than baseline in Group 2 at 6 and 12 months after the initial treatment ($P = 0.012$ and 0.025, respectively). In contrast, the mean BCVA in Group 3 had deteriorated by 12 months after the initial treatment, although it was not significant ($P = 0.12$). The mean central retinal thickness was reduced from baseline after initial treatment in

Table 1 Baseline parameters of participants

	Group 1 (n = 14)	Group 2 (n = 12)	Group 3 (n = 11)	*P* value
Gender (male/female)	11/1	6/4	4/7	0.022†
Age (mean ± SD, years)	82.3 ± 4.1	78.2 ± 5.7	80.3 ± 7.2	0.10*
RAP stage 1	1	3	3	0.61†
Stage 2	6	4	5	
Stage 3	7	5	3	
GLD (mean ± SD)	4737 ± 1704	3948 ± 1238	3291 ± 1418	0.10*
Baseline BCVA logMAR (mean ± SD)	0.82 ± 0.47	0.78 ± 0.55	0.84 ± 0.37	0.84*

Notes: Group 1, PDT monotherapy; Group 2, STA + PDT; Group 3, IVR + PDT; *Kruskal–Wallis test; †Chi-square test.
Abbreviations: GLD, greatest linear dimension; BCVA, best-corrected visual acuity; PDT, photodynamic therapy; STA, subtenon injection of triamcinolone acetonide; IVR, intravitreal injection of ranibizumab; SD, standard deviation.

all groups and the decrease was significant by 12 months in the STA + PDT and IVR + PDT groups (Figure 2). The reduction amplitude was significantly greater in Group 2 than in Group 1 and Group 3 ($P = 0.024$ and $P = 0.033$, respectively, for Group 1 versus Group 2 and Group 2 versus Group 3). In the cases followed up for more than 12 months after initial treatment, the mean number of treatments was significantly lower in Group 2 (1.1 ± 0.4, n = 12) and Group 3 (1.5 ± 0.5, n = 9) than in Group 1 (2.9 ± 0.9, n = 14) over 12 months after initial treatment ($P < 0.0001$, $P = 0.0004$, and $P = 0.15$ for Group 1 versus Group 2, Group 1 versus Group 3 and Group 2 versus Group 3, respectively, Mann–Whitney test) (Figure 3). In Group 2, the mean intraocular pressure before and after STA was 13.8 ± 3.4 mmHg and 16.8 ± 6.1 mmHg, respectively, ($P = 0.18$, two-tailed paired *t*-test). No ocular or systemic complications were found or self-reported in the present cases.

Discussion

We compared the effects of PDT monotherapy, STA + PDT, and IVR + PDT in patients with RAP lesions, and found that the visual outcome was significantly better in those patients who underwent STA + PDT than in those treated with PDT monotherapy, although STA + PDT and IVR + PDT showed no significant difference in visual outcome. The mean number of treatments required per year was significantly lower in the STA + PDT and IVR + PDT groups than for the PDT monotherapy group. In addition, STA + PDT did not cause a significant elevation of intraocular pressure.

Table 2 Transition in best-corrected visual acuity and central retinal thickness of each group

	Group 1	Group 2	Group 3
BCVA (logMAR)			
Baseline	0.82 ± 0.47	0.78 ± 0.55	0.82 ± 0.38
3 months	0.95 ± 0.36	0.66 ± 0.37	0.80 ± 0.44
6 months	1.01 ± 0.31	0.56 ± 0.37*	0.82 ± 0.40
12 months	1.05 ± 0.32	0.57 ± 0.35*	0.81 ± 0.42
CRT (μm)			
Baseline	315 ± 93	358 ± 88	314 ± 102
3 months	268 ± 104	204 ± 72*	211 ± 51*
6 months	260 ± 93	202 ± 84*	223 ± 51*
12 months	263 ± 84	241 ± 74*	202 ± 46*

Notes: Group 1, PDT monotherapy; Group 2, STA + PDT; Group 3, IVR + PDT; *$P < 0.05$ versus baseline.
Abbreviations: CRT, central retinal thickness; BCVA, best corrected visual acuity; PDT, photodynamic therapy; STA, subtenon injection of triamcinolone acetonide; IVR, intravitreal injection of ranibizumab.

Currently, RAP is thought to be the most difficult subtype of exudative AMD to treat.[7] Because previous studies have demonstrated an insufficient effect of PDT monotherapy for RAP lesions,[6,7] most of the recent studies have focused on the effectiveness of PDT combined with IVT or anti-VEGF therapy.[8–11] However, few reports have evaluated the effects of STA + PDT for RAP. Montero et al reported that no better outcomes were observed in RAP patients treated with STA + PDT than in those treated with PDT monotherapy.[13] They administered 40 mg of triamcinolone acetonide immediately after PDT, which resulted in no significant difference in outcomes as compared with PDT monotherapy. However, we used 20 mg of triamcinolone acetonide 4–7 days before PDT, and obtained significantly better outcomes as compared with PDT monotherapy in RAP patients. This difference might be due to the insufficient time for transscleral diffusion of triamcinolone acetonide when it was applied after PDT.[14,15] Rouvas et al reported favorable outcomes for IVT + PDT when IVT was performed 7 ± 3 days before PDT.[10] The mechanism by which triamcinolone acetonide works to improve the outcome of PDT is still a matter of speculation. An inflammatory response and upregulation of VEGF have been reported after application of PDT.[17,18] Because triamcinolone acetonide has antiangiogenic, anti-inflammatory, and anti-VEGF effects,[19,20] the combination of PDT and triamcinolone acetonide may reduce the inflammatory response and upregulation of VEGF associated with choroidal neovascularization and PDT.

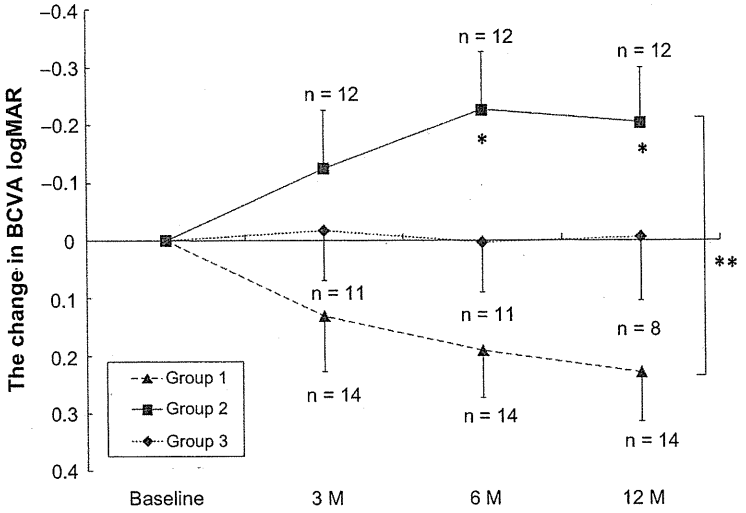

Figure 1 Changes in the BCVA of RAP patients after PDT, STA + PDT, and IVR + PDT. The BCVA was determined using the Landolt C chart, and is presented as decimal visual acuities. Triangles with dashed line: PDT (Group 1); squares with solid line: STA + PDT (Group 2); diamonds with dot line: IVR + PDT (Group 3).
Notes: Values represent mean ± SEM; *$P < 0.05$ compared to baseline; **$P < 0.05$ between Group 1 and Group 2.
Abbreviations: BCVA, best corrected visual acuity; IVR, intravitreal injections of ranibizumab; RAP, retinal angiomatous proliferation; PDT, photodynamic therapy, STA, subtenon injections of triamcinolone acetonide.

The significantly greater central retinal thickness reduction after STA + PDT than after IVR + PDT in the present study might reflect a difference in anti-inflammatory and anti-VEGF effects between STA and IVR, which was possibly associated with the better outcome, although not significant, in the post-treatment BCVA for the STA + PDT group than for the IVR + PDT group. Although we performed IVR 3–7 days before PDT, the timing of IVR might be too early because Debefe et al reported that ranibizumab should be administered within 24 hours after PDT in accordance with their experimental results.[21] A recent report showed favorable visual outcomes after IVR + PDT when PDT was performed 1–2 days after IVR.[22] In addition, unlike previous reports, we did not add two extra monthly IVR after PDT in Group 3 to save treatment costs, which might reduce the effect of IVR + PDT. However, the change in logMAR BCVA in Group 3 was almost equivalent to that of the previous report (0.02 between baseline and at least 6 months after the first therapy) performing three IVR + one PDT as an initial treatment.[10] There is another possibility that the effects of

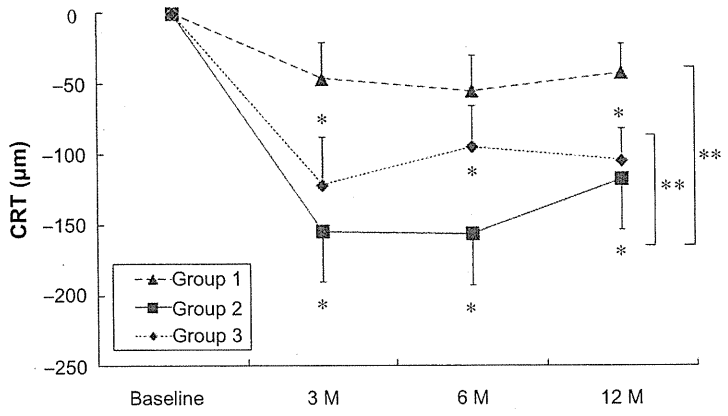

Figure 2 Changes in the CRT of RAP patients after PDT, STA + PDT, and IVR + PDT. Triangles with dashed line: PDT (Group 1); squares with solid line: STA + PDT (Group 2); diamonds with dot line: IVR + PDT (Group 3).
Notes: Values represent mean ± SEM. *$P < 0.05$. **$P < 0.05$ between Group 1 and Group 2, or between Group 2 and Group 3.
Abbreviations: CRT, central retinal thickness; IVR, intravitreal injections of ranibizumab; RAP, retinal angiomatous proliferation; PDT, photodynamic therapy, STA, subtenon injections of triamcinolone acetonide.

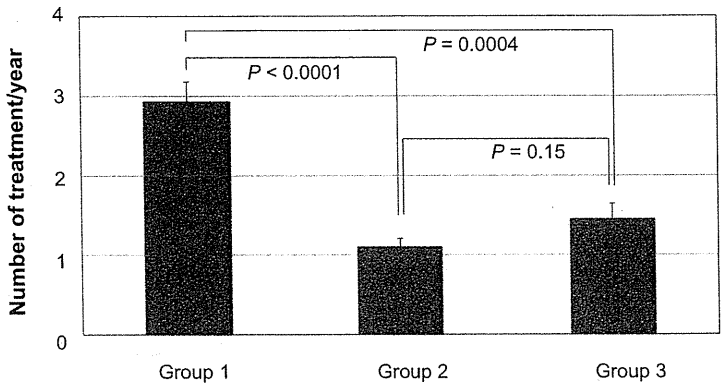

Figure 3 Number of treatments performed in 12 months.
Note: Values represent mean + SEM.

STA remained for several months after PDT and inhibited regrowth of neovascular tracts, reducing the number of treatments required to suppress the RAP lesions, and thus resulted in reduced cumulative retinal damage caused by PDT. Because a single STA is thought to have antiangiogenic and anti-inflammatory effects lasting up to 3 months[23] while a single dose of IVR can work for a month, STA + PDT might have an advantage to suppress the RAP lesion longer than IVR + PDT. Conversely, IVR + PDT may necessitate some additional IVR during follow-up period.

Our results showed that the best mean BCVA was obtained at 6 months after the initial STA + PDT, but this was reduced by 12 months, mainly due to reactivation of RAP lesions in some cases. It is interesting that a reactivation of RAP lesions at 6 months after treatment was previously reported in cases treated with IVT + PDT.[24] A previous review article mentioned that the best improvement in BCVA was achieved at 6 months after initial PDT + IVT, and that the effects faded by 12 months, with a high incidence of cataracts.[9] However, in our study, no patient in the STA + PDT group showed any progression of cataracts during the follow-up period. In addition, the incidence of intraocular pressure elevation was reported less often with STA than with IVT.[25]

Currently, many treatment procedures are being tested and compared to establish the best strategy for treating RAP lesions. Among them, anti-VEGF therapy is the most investigated modality which could be applied alone[26,27] or combined with PDT.[10,11,28] However, IVR + PDT is likely to be a very expensive therapy and intravitreal bevacizumab + PDT is not possible without off-label use of bevacizumab under current circumstances. If STA + PDT showed similar or better outcomes to PDT+ anti-VEGF therapy or anti-VEGF monotherapy, there is a greater cost-effectiveness for patients. In fact, the improvement in BCVA (–0.22 ± 0.34 logMAR units) with STA + PDT in this study was almost equivalent to the average of previous reports (–0.17 ± 0.12 logMAR units) with anti-VEGF monotherapy.[9,29]

The major limitations of the present study were its non-randomized and retrospective nature and the small number of subjects. Hence, it is important to evaluate the results of a randomized controlled trial for STA + PDT with a larger number of subjects to determine the efficacy of this therapy, particularly against RAP. Therefore, further investigation will be needed to determine the correct interventions for RAP.

In conclusion, STA + PDT may be an effective therapy for RAP lesions during the first 12 months after treatment, although the effects need to be further evaluated.

Acknowledgments

This study was supported by a grant-in aid (23592567) from the Ministry of Education, Science, and Culture, Tokyo, Japan (SH), and by a grant from the Takeda Science Foundation (SH).

Disclosure

No authors have any financial or conflicting interests to disclose in this work.

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Molecular Vision 2012; 18:121-127 <http://www.molvis.org/molvis/v18/a15>
Received 19 October 2011 | Accepted 12 January 2012 | Published 18 January 2012

The association of *CD36* variants with polypoidal choroidal vasculopathy compared to typical neovascular age-related macular degeneration

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Purpose: To clarify the association of cluster of differentiation 36 (*CD36*) variants with polypoidal choroidal vasculopathy (PCV) and compare them with those in typical neovascular age-related macular degeneration (tAMD).
Methods: We included 349 Japanese AMD patients (210 PCV, 139 tAMD) and 198 age-matched controls. Four tag single-nucleotide polymorphisms (SNPs)—rs10499862, rs3173798, rs3211883, and rs3173800—in the *CD36* region were genotyped using the TaqMan assay. Allelic and genotypic frequencies of the SNPs were tested.
Results: Although none of the SNPs tested were associated with PCV, the allelic frequencies of rs3173798 and rs3173800 were significantly different between PCV and tAMD patients. Genotype association analysis demonstrated different associations of these two SNPs between PCV and tAMD in the genotype model. Haplotype analysis revealed that the association of the major haplotype (T-T-T-T) at four selected SNPs in CD36 differed significantly between PCV and tAMD patients.
Conclusions: The *CD36* region may be associated with the difference in genetic susceptibility for PCV and tAMD.

Age-related macular degeneration (AMD) is a leading cause of central vision loss in the elderly in industrialized countries [1]. Although the number of patients with AMD has increased remarkably over the years, the pathology of AMD is not well understood [2]. Advanced AMD is clinically classified into atrophic AMD and exudative AMD. In exudative AMD, there are two characteristic phenotypes distinct from typical neovascular AMD (tAMD), which are called polypoidal choroidal vasculopathy (PCV) [3,4] and retinal angiomatous proliferation [5]. These two phenotypes are known to have different aspects from tAMD in their natural courses, as well as different responses to interventions such as photodynamic therapy (PDT) and anti–vascular endothelial growth factor therapy, although the reasons for this remain unknown [6-10]. Recently, several genetic association studies have been conducted to explain the different characteristics among the phenotypes of exudative AMD and their susceptibility to several interventions, mainly PDT [11-19].

Cluster of differentiation 36 (CD36) is a multifunctional molecule that plays an important role in lipid metabolism, angiogenesis, inflammation, and scavenging oxidative stresses [20-22], all of which may be involved in the pathogenesis of AMD and in the mechanism whereby PDT functions. We previously demonstrated the association of coding variants in the *CD36* region with the incidence of neovascular AMD (corresponding to tAMD in the present report) in the Japanese population [23]. In that study, two variants of single nucleotide polymorphisms (SNPs)—rs3173798 and rs3211883 on introns 3 and 4, which have high linkage disequilibrium—showed the greatest association with susceptibility to neovascular AMD. However, it has not been determined whether this association is general to all exudative AMD or specific for tAMD. We hypothesized that the genetic variants in *CD36* may be differently associated with genetic susceptibilities to tAMD and PCV. Since PCV is known to have a better response to PDT than tAMD [7,8], we considered that the scavenging ability of CD36 for reactive oxygen species generated by PDT might be different between tAMD and PCV, which could influence the effect of PDT.

We previously reported that coding variants of the elastin gene (*ELN*) were associated with PCV but not with tAMD [13]. However, two recent reports with larger cohorts showed the opposite results: The *ELN* polymorphism was associated with tAMD but not with PCV [24,25]. Although the association of elastin gene variants with tAMD and PCV is still inconclusive, these results might have been generated due to statistical type 1 and type 2 errors. In the present study, we included a sufficient number of subjects based on power analysis to prevent these errors.

In this study, we genotyped four tag SNPs located on introns 3 and 4 of the *CD36* gene, and analyzed the association between these variants and the incidence of PCV, as well as tAMD, in a Japanese population.

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TABLE 1. DATA SUMMARY OF PCV AND TAMD PATIENTS AND CONTROL SUBJECTS.

Factors	PCV	tAMD	Control
Number of subjects	210	139	198
Gender (male/female)	166/44	108/31	119/79
Mean age±SD (years)	73.8±7.5	75.3±7.3	72.1±5.9
Median age (years)	75	76	72
Age range (years)	51–93	55–94	56–95

PCV: polypoidal choroidal vasculopathy, tAMD: typical neovascular AMD.

METHODS

Study participants: This is an extension study of a previous report [23]. The study was approved by the Institutional Review Board of the Kobe University Graduate School of Medicine, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. All cases in this study were Japanese individuals recruited from the Department of Ophthalmology at Kobe University Hospital in Japan.

The study included 349 Japanese AMD patients (210 PCV, 139 tAMD) and 198 age-matched controls who accepted DNA sampling. The tAMD group in this study included the patients of our previous study, along with 30 new patients. The control group in this study included the subjects of the previous study and 16 more individuals [23]. All patients received ophthalmic examinations, including visual acuity measurements with refractive correction, slit-lamp biomicroscopy of the fundi, color fundus photographs, optical coherence tomography, fluorescein angiography, and indocyanine green angiography (ICGA). All PCV subjects enrolled in this study met the criteria of definite cases of PCV as proposed by the Japanese Study Group of Polypoidal Choroidal Vasculopathy [26]. ICGA showed a choroidal origin of the polypoidal lesions in all PCV cases, typically with vascular networks in the posterior poles and subretinal reddish-orange protrusions corresponding to the polypoidal lesions on ICGA. In contrast, all tAMD patients had clear images of choroidal neovascular networks on ICGA. The classification of the AMD phenotype was performed by three independent retinal specialists for each case under masked conditions for the genotype. Only those cases whose diagnoses were matched by all three readers were included in this study. The details of the participants are listed in Table 1.

Single-nucleotide polymorphism selection: The four tag SNPs —rs10499862, rs3173798, rs3211883, and rs3173800 on introns 3 and 4 in the *CD36* region—were selected based on our previous study [23], including SNPs which were significantly associated with tAMD (allelic nominal p-values <0.01). These four SNPs were in a single haplotype block using the algorithm based on the solid spine of linkage disequilibrium.

Genotyping: Genomic DNA was extracted from the peripheral blood using the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA). Genotyping was performed using TaqMan® SNP Genotyping Assays or Custom TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) in accordance with the supplier's recommendations.

Statistical analysis: All SNPs were evaluated for Hardy–Weinberg equilibrium using the χ^2 test (one degree of freedom) with SNPAlyze version 7.0.1 (DYNACOM, Yokohama, Japan). The allelic and genotype frequency distributions were compared among tAMD, PCV, and control subjects using a χ^2 test with one or two degrees of freedom for the allelic and genotypic tests, respectively. To avoid false-positive results, the Bonferroni or permutation correction was added for each comparison. P values <0.05 were considered to be statistically significant.

To exclude a potential stratification, our study cohort was examined by Structure 2.0 [27] using 26 unlinked genome-wide SNPs, as shown in our previous report [23].

RESULTS

None of the SNPs reported in the present study showed any significant deviations from the Hardy–Weinberg equilibrium over the entire sample (p>0.05). Table 2 summarizes the minor allelic frequencies for all SNPs and the results from a single-SNP association study. Significant differences in minor allelic frequencies were found in all SNPs tested between the tAMD and control subjects, as shown in our previous report [23], but there was no difference in minor allelic frequencies between the PCV and control subjects. Meanwhile, significant differences were found between PCV and tAMD patients at rs3173798 and rs3173800. The statistical powers of single-SNP association analysis at rs3173798 and rs3173800 were about 0.94 and 0.92, respectively (alpha error <0.05), in the comparison between PCV and tAMD. The genotype association analysis revealed significant associations of rs3173798 and rs3211883 with tAMD, but not with PCV in the dominant model (Table 3). In addition, the most significant differences were found between PCV and tAMD patients at rs3173798 and rs3173800 in the

TABLE 2. SUMMARY OF SINGLE-SNP ASSOCIATION ANALYSIS ON GENE *CD36*.

SNP ID	Location	Major/ Minor	Minor allele frequency			Allelic nominal p-value (Bonferroni p-value)		
			PCV	tAMD	Control	PCV versus control	tAMD versus control	PCV versus tAMD
rs10499862	Intron 3	T/C	0.14	0.11	0.18	0.058 (0.23)	0.0066 (0.026)	0.28 (1.0)
rs3173798	Intron 3	T/C	0.46	0.33	0.44	0.57 (1.0)	0.005 (0.020)	0.00081 (0.0032)
rs3211883	Intron 4	T/A	0.35	0.28	0.4	0.19 (0.76)	0.0017 (0.0068)	0.043 (0.17)
rs3173800	Intron 4	A/T	0.26	0.38	0.28	0.41 (1.0)	0.0071 (0.028)	0.0005 (0.0020)

SNP: single nucleotide polymorphism, PCV: polypoidal choroidal vasculopathy, tAMD: typical neovascular AMD.

genotype (additive) model. In the haplotype analysis, the most frequent haplotype (T-T-T-T) at rs10499862, rs3173798, rs3211883, and rs3173800 showed the most significant difference in association between PCV and tAMD (Table 4). There was no evidence of significant stratification in our study cohort (*Pr* [*K*=1 >0.99]).

DISCUSSION

We genotyped four tag SNPs in the *CD36* region, and found that the minor allelic frequencies at SNPs rs3173798 and rs3173800 and the haplotype at four selected SNPs in the *CD36* region were significantly associated with the difference in genetic susceptibility to PCV and tAMD. Namely, T, T alleles were less frequent than C, A alleles in PCV than in tAMD patients at rs3173798 and rs3173800, respectively. Moreover, the T-T-T-T haplotype at rs10499862, rs3173798, rs3211883, and rs3173800 on introns 3 and 4 in the *CD36* region was significantly less frequent in PCV than in tAMD.

Although our previous report demonstrated an association of SNPs in the *CD36* region with neovascular AMD [23], the phenotype specificity of these SNPs remained to be elucidated. The present study suggests a different association of *CD36* in genetic susceptibility for PCV and tAMD, which may contribute to the different clinical characteristics of PCV (i.e., natural course and the response to PDT or ranibizumab) from tAMD [3,4,6-9]. Since the SNPs at rs10499862,rs3173798,rs3211883, and rs3173800 were not covered by the gene chips used in previous genome-wide association studies [28,29], they could not be detected as possible causative SNPs for AMD in those studies. The present study, as well as our previous report [23], further suggested an association of this region in *CD36* with the incidence of tAMD. However, these SNPs did not remain significant in the prevalence of PCV. Moreover, a statistically significant difference was detected in the association of this region between tAMD and PCV. This suggests a different association of the *CD36* region with the phenotype of neovascular AMD, although the details have not yet been clarified. A recent in vivo study demonstrated that a downregulation of *CD36* in capillary sprout endothelial cells facilitated angiogenesis [30]. Rats carrying a specific genetic variant of *CD36* have been found to be more susceptible to light-induced retinal damage, and are more likely to develop age-related retinal degeneration and choriocapillary rarefaction [31]. CD36 is involved in diverse physiologic and pathological processes, including scavenger receptor functions, transforming growth factor-β activation, lipid metabolism, angiogenesis, atherogenesis, and inflammation, depending on the ligands with which CD36 can interact [20-22]. In particular, the scavenging ability of CD36 for oxidative stress is critical to manage AMD, since oxidative stress is widely recognized as an important component in the pathogenesis of AMD [32,33] and in the mechanism whereby PDT works to occlude neovascular tracts [34]. A recent in vitro study reported that the uptake of oxidized low-density lipoprotein (oxLDL) induces the expression of several genes related to oxidative stress, inflammation, and apoptosis in retinal pigment epithelium cells [35]. An immunohistochemical study reported the presence of oxLDL in surgically excised choroidal neovascularization membranes [36]. Moreover, the verteporfin used in PDT binds with serum LDL, and this complex is incorporated into choroidal neovascularization tissues [37]. Although it is not known whether lipid metabolism and oxidative stress play different roles in the pathogenesis between tAMD and PCV, the present study implied different genetic susceptibilities for each AMD phenotype. Interestingly, recent reports demonstrated a difference between tAMD and PCV in their histological findings and systemic risk factors [38,39]. Moreover, our previous study demonstrated a different association of an elastin gene polymorphism with tAMD and PCV [13].

The biologic basis of the association with the haplotype in *CD36* is currently unknown, because the haplotype in the present study does not reside in the coding sequence of *CD36*. FASTSNP [40] gave the information that rs3173798 is located at splicing site with medium-high effect, but it was not shown whether it is located at splice donor site or acceptor