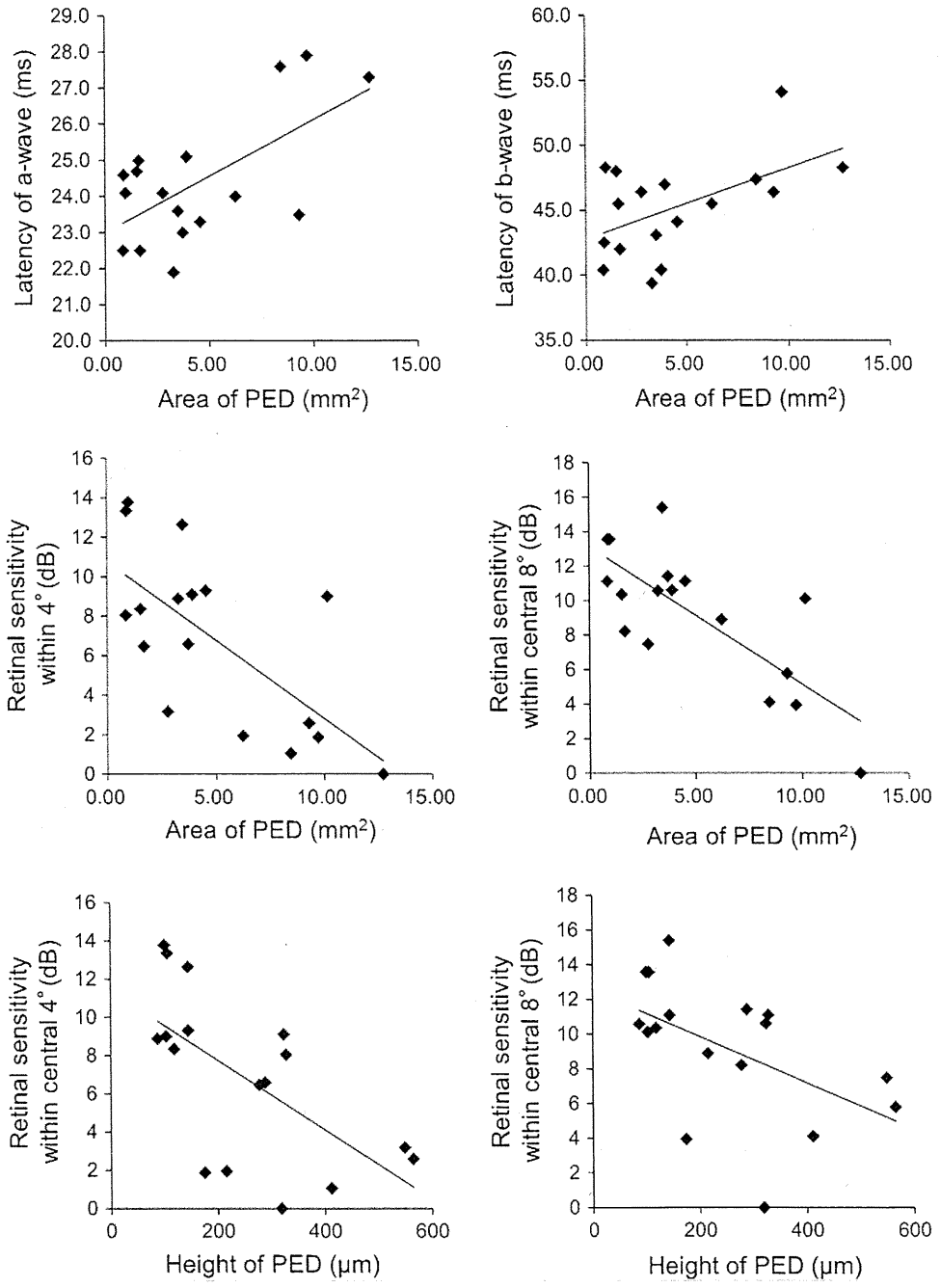

Fig. 7 Scattergram of the size of the drusenoid pigment epithelial detachment and macular functions measured with focal macular electroretinography or microperimetry. *PED* pigment epithelium detachment

antivascular endothelial growth factor therapy [40–43]. Gallego-Pinazo et al. [41] successfully treated six patients with drusenoid PED using intravitreal ranibizumab. However, Krishnan and Lochhead [42] reported rapid development of geographic atrophy after intravitreal injection of pegaptanib in an eye with drusenoid PED. In a recent report from the Age-Related Eye Disease Study, 19 % of eyes with drusenoid PED developed central geographic atrophy, and 23 % of these developed neovascular AMD [35]. When geographic atrophy develops in the extrafoveal region, VA measurement does not reflect a visual disturbance. The effect of treatment for drusenoid PED remains controversial. Multimodal measurements of macular function would be most helpful to evaluate the treatment efficacy of drusenoid PED.

There are various limitations to the current study. First, the eligible patients and controls in this study were all Japanese, and the genetic background may well have influenced the characteristics of AMD, so our results should be confirmed in another population. Second, the sample size of each group was small, so it is possible that we did not detect small differences between groups. Third,

the current study excluded central geographic atrophy, primarily because this is a relatively rare feature of AMD in Japanese patients. Finally, this was a cross-sectional study, so we could not offer any information regarding changes in macular function over time. Further longitudinal studies are necessary to fully elucidate the macular function in eyes with AMD of various stages and to study the treatment effects and the natural course of eyes with AMD, especially those with AMD in the early stage. Multimodal evaluations of the entire macular function should be of great help in these endeavors.

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Association Between *ZIC2*, *RASGRF1*, and *SHISA6* Genes and High Myopia in Japanese Subjects

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Purpose. We investigated the association of genetic variations, which were identified recently in a large-scale genome-wide association study (GWAS) to confer risk of refractive error and common myopia in Caucasians, with high myopia in Japanese subjects.

Methods. The 5 single-nucleotide polymorphisms (SNPs) from the 5 genes *TOX*, *RDH5*, *ZIC2*, *RASGRF1*, and *SHISA6*, were genotyped in 1339 unrelated highly myopic Japanese patients and 3248 healthy Japanese participants in the Nagahama Study. In addition, genotypes were compared between high myopia patients without choroidal neovascularization (CNV) and patients with myopic CNV.

Results. Significant associations between rs8000973 near *ZIC2* ($P = 7.16 \times 10^{-7}$), rs4778879 in *RASGRF1* ($P = 3.40 \times 10^{-7}$), and rs2969180 in *SHISA6* ($P = 0.033$) and high myopia were observed. Odds ratios (95% confidence intervals) were 1.33 (1.19-1.49), 0.78 (0.71-0.86), and 1.11 (1.01-1.22) for the rs8000973 C allele, rs4778879 A allele, and rs2969180 G allele, respectively. The effect of the rs2969180 allele G contrasted with that observed in the original report, whereas the effect of the other 2 SNPs agreed. Further analysis using controls with -1.0 diopter (D) $\leq$ spherical equivalent $\leq +1.0$ D showed a significant association between *ZIC2* and *RASGRF1*, but not *SHISA6*. Among the patients with high myopia, 516 had myopic CNV in either eye, while 823 patients did not have myopic CNV in eyes. No evaluated genes showed a significant association with the development of myopic CNV.

Conclusions. *ZIC2* and *RASGRF1* are susceptibility genes, not only for common myopia, but also for high myopia.

Keywords: high myopia, ZIC2, RASGRF1, SHISA6, CNV

Myopia, or nearsightedness, is the most common ocular disorder worldwide. Recent studies reported that the prevalence of myopia is approximately 20% to 42% in the Caucasian population, and much higher (40%-70%) in East Asian populations.[1-4] High myopia is distinguished from common myopia by an excessive increase in the axial length of the eye[5,6] and is considered important because of its association with various ocular complications that lead to blindness.[7-10] For example, choroidal neovascularization (CNV) beneath the fovea is one of the most vision-threatening complications of high myopia.[11,12]

Previous studies have indicated the involvement of genetic and environmental factors in the progression of myopia.[13-16] Family-based linkage analyses and twin studies have identified MYP1-19 loci and several candidate genes,[17,18] but genetic screening studies have achieved limited success. Since 2009, several genome-wide association studies (GWAS) have reported candidate genes for myopia,[19-26] but none of the reported genes or loci, except for the 15q14 locus, showed a consistent association with either common or high myopia in later studies.[27-30] Moreover, although some loci were reported to be associated with common and high myopia,[25,27,31] it still is not clear whether common myopia and high myopia share the same genetic background.

Recently, Verhoeven et al.[32] and Kiefer et al.[33] conducted a large-scale GWAS independently, and reported multiple new susceptibility loci for refractive error and common myopia. To investigate whether these loci cause high myopia in Japanese subjects, we performed a large-scale, case-control study on high myopia. In addition, we investigated the contribution of these genetic variations to the occurrence of CNV in high myopic eyes.

Methods

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

Table 1. Characteristics of the Study Population

	Patients, High Myopia*	Controls†
Patients, *n*	1339	3248
Age in y, mean ± SD	57.13 ± 14.90	52.20 ± 14.12
Sex, *n* (%)		
Male	442 (33.0%)	1092 (33.6%)
Female	897 (67.0%)	2154 (66.4%)
Axial length, mm ± SD		
Right eyes	29.25 ± 1.87	24.11 ± 1.39
Left eyes	29.12 ± 1.83	24.07 ± 1.39
Refraction of the phakic eyes, D‡		
Right eyes	−12.39 ± 4.66	−1.73 ± 2.85
Left eyes	−12.54 ± 4.59	−1.64 ± 2.80

* Axial length of ≥26.0 mm in eyes.

† Healthy individuals recruited from Nagaha cohort study.

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

Patients and Controls

A total of 1339 unrelated highly myopic Japanese patients was recruited from Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, Kobe City Medical Center General Hospital, Ozaki Eye Hospital, and Otsu Red-Cross Hospital. All patients underwent comprehensive ophthalmic examinations, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography (UD-6000; Tomey, Nagoya, Japan) or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA). Patients with an axial length of ≥26.0 mm in both eyes were placed into the high myopia group. For control subjects, we included 3248 unrelated healthy Japanese subjects (control 1) from the Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience dataset (The Nagahama Study). Automatic objective refraction and measurements of the axial length by partial coherence interferometry (IOLMaster; Carl Zeiss Meditec) were performed on all participants. For subanalysis, subjects with a spherical equivalent between −1.0 and +1.0 diopters (D) in both eyes also were included as a control group (control 2). All participants were Japanese, and subjects with any history of ocular disease were eliminated from the control group.

To evaluate the contribution of single-nucleotide polymorphisms (SNPs) to the occurrence of CNV in myopic eyes, the high myopia group was divided into 2 groups: CNV and no CNV. The inclusion criteria for the CNV group were clinical presentation and angiographic manifestations of macular CNV or Fuchs' spot in at least 1 eye.

SNP Selection

Verhoeven et al.[32] reported 26 loci (29 potential candidate genes) associated with refractive error and common myopia in a large-scale multi-ethnic GWAS. Of these loci, 8 also were reported to be associated with myopia and replicated in another recent large-scale GWAS including Caucasian participants.[33] For our analysis, we selected 8 SNPs in these 8 loci that were evaluated in the original report. Among these 8 SNPs, 3 showed extremely low minor allele frequency (MAF) in the Japanese population according to the HapMap data (rs12205363 in *LAMA2*, rs1656404 near *PRSS56*, and rs1960445 near *BMP3*; MAF 0.00, 0.01, and 0.02, respectively). In addition to these 3 SNPs, rs524952 in *GJD2* also was excluded as we had confirmed its association previously with high myopia.[27] Selected SNPs included rs7837791 near *TOX*, rs3138144 in *RDH5*, rs8000973 near *ZIC2*, and rs2969180 in *SHISA6*. Although negated by Kiefer et al.[33] at the replication stage ($P = 0.08$), rs4778879 in *RASGRF1* was included because its association with myopia still is disputed despite numerous replication studies.

Genotyping

Genomic DNAs were prepared from peripheral blood by using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). Genotyping of samples from 1339 high myopic patients was performed using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). For the control group, 3712 individuals from the Nagahama study were genotyped using HumanHap610K Quad Arrays, HumanOmni2.5M Arrays, and/or HumanExome Arrays (Illumina, Inc., San Diego, CA). To ensure high-quality genotype data, a series of quality control (QC) filters were applied to the data from each platform, including MAF cutoffs (MAF > 0.01), Hardy-Weinberg equilibrium (HWE; $P > 1 \times 10^{-7}$), genotypic success rate (>95%), individual call rate (>99%), and estimated relatedness (PI-HAT < 0.35). The QCs were performed using PLINK (ver.1.07; available in the public domain at http://pngu.mgh.harvard.edu/purcell/plink/). The fixed dataset consisted of 3248 individuals. Genotype data directly assessed by arrays was used for analyses. Because directly genotyped data of SNP rs4778879 in *RASGRF1* in controls was not available, we analyzed genotype counts of SNP rs6495367 whose linkage disequilibrium value (r^2) is 1.0 compared to rs4778879 (HapMap phase II + III rel 28 JPT).

Statistical Analyses

Data are presented as the mean ± SD. Deviations in the genotype distribution from the HWE were assessed for each group by using the HWE exact test. The χ^2 test for the trend or its exact counterpart was used to compare the genotype distribution of 2 groups. To adjust for age and sex, we performed multiple regression and logistic regression analyses. Two subjects in the control group were excluded from multiple regression and logistic regression analyses because of lack of information regarding age or sex. Statistical analyses were performed using SPSS software (version 21.0; SPSS Science, Chicago, IL). A P value of <0.05 was considered statistically significant. To analyze CNV, a P value of <0.01 (= 0.05/5) was considered statistically significant after Bonferroni correction. Power calculations were performed using R software, package "pwr" (v 3.0.0; R Foundation for Statistical Computing, Vienna, Austria; available in the public domain at http://www.r-project.org/).

Results

Basic information of the study population is shown in Table 1. The mean age of the 1339 high myopia cases was 57.13 ± 14.90 years and the male-to-female ratio was 33.0%:67.0%. The average axial length of cases was 29.19 ± 1.85 mm. Among the 2678 eyes included in the study, 1920 (71.7%) were phakic, and the mean refraction of the phakic eyes was −12.68 ± 4.54 D. The mean age of the 3248 control subjects was 52.20 ± 14.12 years, and the male-to-female ratio was 33.6%:66.4%. The average axial length of controls was 24.09 ± 1.39 mm, and the mean refraction of the 5572 (85.8%) phakic eyes was −1.68 ±

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Table 2. Genotype Frequency, Associations, and Odds Ratios (ORs) in the High Myopia Patients and Controls (Control 1)

				Genotype Frequency								
SNP	Chr	Position	Genes	Genotype	High Myopia	Control 1	Nominal *P**	Adjusted *P*†	Adjusted OR†	95% CI†	N‡	HWE *P*§
rs7837791	8	60179086	*TOX*	GG	22.1%	21.7%	0.47	0.62	1.02	0.93–1.12	3239	0.76
				TG	50.9%	50.0%						
				TT	27.0%	28.3%						
rs3138144	12	56114769	*RDH5*	CC	19.2%	20.9%	0.41	0.28	0.95	0.85–1.05	1848	0.49
				CG	50.1%	48.7%						
				GG	30.7%	30.4%						
rs8000973	13	100691367	*ZIC2*	CC	10.2%	6.5%	8.64E-07	7.16E-07	1.33	1.19–1.49	1849	0.76
				TC	42.8%	38.6%						
				TT	47.0%	54.8%						
rs4778879	15	79372875	*RASGRF1*	AA	17.9%	(GG) 23.5%‖	1.46E-07	3.40E-07	0.78	0.71–0.86	3244‖	0.88‖
				GA	49.0%	(GA) 50.1%‖						
				GG	33.1%	(AA) 26.4%‖						
rs2969180	17	11407901	*SHISA6*	GG	24.5%	20.7%	0.023	0.033	1.11	1.01–1.22	3240	0.10
				AG	49.0%	51.2%						
				AA	26.5%	28.1%						

Chr, chromosome; CI, confidence interval.
* Differences in the observed genotypic distribution were examined by χ^2 test for trend.
† Age and sex adjustment was performed based on a logistic regression model.
‡ Number of control subjects who were genotyped directly.
§ The HWE test results for control subjects who were genotyped directly.
‖ Data of SNP rs6495367, whose linkage disequilibrium value (r^2) is 1.0 compared to SNP rs4778879.

2.82 D. Among the control group, 999 subjects had a spherical equivalent between −1.0 and +1.0 D in both eyes, and these subjects were used as control 2. Their average axial length was 23.38 ± 0.79 mm, and the mean refraction of the 1998 (100%) phakic eyes was −0.11 ± 0.53 D.

Genotype counts, associations examined using the χ^2 test for trend analysis, odds ratios for the 5 SNPs between high myopia cases and controls, number of control subjects who were genotyped directly, and the results of the HWE exact test in controls are shown in Table 2. The SNPs rs8000973 near *ZIC2*, rs4778879 in *RASGRF1*, and rs2969180 in *SHISA6* showed significant association with high myopia ($P = 7.16 \times 10^{-7}$, 3.40×10^{-7}, and 0.033, respectively). The odds ratios (95% confidence intervals) were 1.33 (1.19–1.49) for the rs8000973 C allele, 0.78 (0.71–0.86) for the rs4778879 A allele, and 1.11 (1.01–1.22) for the rs2969180 G allele. The effect of the rs2969180 allele G was contrasting to that obtained in the previous study, whereas the other 2 SNPs showed the same trend as that observed in the original report. The distributions of the genotypes for all the five SNPs were in HWE. When control group 2 was evaluated, the SNPs rs8000973 and rs4778879 showed significant association with high myopia

Table 3. Genotype Frequency, Associations, and ORs in the High Myopia Patients and Control 2

				Genotype frequency							
SNP	Chr	Position	Genes	Genotype	High Myopia	Control 2*	Nominal *P*†	Adjusted *P*‡	Adjusted OR‡	95% CI‡	N§
rs7837791	8	60179086	*TOX*	GG	22.1%	20.7%	0.20	0.24	1.07	0.95–1.21	997
				TG	50.9%	49.9%					
				TT	27.0%	29.4%					
rs3138144	12	56114769	*RDH5*	CC	19.2%	20.5%	0.26	0.21	0.91	0.79–1.05	567
				CG	50.1%	51.5%					
				GG	30.7%	28.0%					
rs8000973	13	100691367	*ZIC2*	CC	10.2%	7.0%	1.33E-05	1.29E-05	1.43	1.22–1.67	568
				TC	42.8%	34.7%					
				TT	47.0%	58.3%					
rs4778879	15	79372875	*RASGRF1*	AA	17.9%	(GG) 26.4%‖	1.28E-07	1.01E-07	0.72	0.64–0.82	998‖
				GA	49.0%	(GA) 47.8%‖					
				GG	33.1%	(AA) 25.8%‖					
rs2969180	17	11407901	*SHISA6*	GG	24.5%	21.2%	0.043	0.076	1.11	0.99–1.25	996
				AG	49.0%	49.5%					
				AA	26.5%	29.3%					

* Healthy individuals with spherical equivalent between −1.00 and +1.00 in eyes.
† Differences in the observed genotypic distribution were examined by χ^2 test for trend.
‡ Age and sex adjustment was performed based on a logistic regression model.
§ Number of control subjects who were genotyped directly.
‖ Data of SNP rs6495367 whose linkage disequilibrium value (r^2) is 1.0 compared to SNP rs4778879.

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TABLE 4. Characteristics of the High Myopic Patients With CNV and With No CNV

	CNV	No CNV
Patients, *n*	516	823
Age in y, mean ± SD	60.99 ± 13.28	54.56 ± 15.56
Sex, *n* (%)		
Male	112 (21.7%)	330 (40.1%)
Female	404 (78.3%)	493 (59.9%)
Axial length, mm ± SD		
Right eyes	29.29 ± 1.71	29.22 ± 1.96
Left eyes	29.10 ± 1.69	29.13 ± 1.91

(Table 3, $P = 1.29 \times 10^{-5}$ and 1.01×10^{-7}, respectively). In contrast, rs2969180 in *SHISA6* showed a marginal association with high myopia (nominal $P = 0.043$ and adjusted $P = 0.076$). The SNPs in *RDH5* (rs3138144) and near *TOX* (rs7837791) showed no association with high myopia for all settings examined in this study.

Among the 1339 high myopic patients, 516 had CNV, while 823 did not. The demographics of the CNV group and the no CNV group are shown in Table 4. There was no difference in the axial lengths in each group ($P > 0.05$), whereas the age and female ratios were significantly higher in the CNV group ($P < 0.05$), as was reported previously.[12,34] The results of the association between the genetic variants and myopic CNV in this study are shown in Table 5. None of these 5 SNPs showed significant associations with CNV occurrence in the high myopia patients after Bonferroni correction.

DISCUSSION

In the present study, we showed that SNPs rs8000973 near *ZIC2* and rs4778879 in *RASGRF1*, which were reported recently as susceptibility loci for common myopia, were significantly associated with high myopia in Japanese subjects. Our study also suggested that rs2969180 in *SHISA6* is associated with high myopia. Although it is unclear whether common and high myopia share the same genetic background, our results indicated the existence of some overlap.

The association between the 15q25 locus/*RASGRF1* region and myopia still is controversial; however, our findings strongly suggested the contribution of the 15q25 locus/*RASGRF1* region to high myopia. The 15q25 locus/*RASGRF1* region was reported initially by Hysi et al.[21] to be associated with refractive error and common myopia in a large-scale GWAS by using Caucasian cohorts. However, later studies could not replicate its association with common myopia,[28-30] and its association with high myopia remains controversial. We showed that this locus had a weak association ($P = 0.031$ for rs8027411 and $P = 0.047$ for rs17175798) with high myopia in Japanese subjects,[27] but a Chinese study showed no association of 15q25 with moderate or high myopia. In contrast with these 2 reports on high myopia, our study used a larger number of cases and a larger control group, which differed from that used in our previous study, and the examined SNP also was different from those in previous reports. Because rs4778879 showed weak linkage disequilibrium with previously investigated SNPs, the number of samples would lead to the contradictory results obtained for high myopia between the present and previous studies. Further study on common myopia by using a relatively larger number of samples may confirm the association between the 15q25 locus/*RASGRF1* region and common myopia.

The risk allele in rs8000973 near *ZIC2* and rs4778879 in *RASGRF1* was the same as that observed in the previous study, but the effect of rs2969180 in *SHISA6* differed from that observed in the previous study. Of the SNPs examined in this study, the MAFs in the control group and those obtained from the HapMap data were fairly consistent. The significance of the association of *SHISA6* was weaker than that of *ZIC2* and *RASGRF1* when compared with the population controls (control 1), and it was marginal when compared with the subjects with emmetropic refractive error (−1.0 to +1.0 D) in eyes (control 2). In control 1, the average axial length and mean refraction of the phakic eyes were slightly shifted to a myopic range (24.09 ± 1.39 mm, and −1.68 ± 2.82 D, respectively) as a logical outcome of the high prevalence of myopia (40%–70%) in the Japanese population. Because control 1 included high myopia participants, as the Japanese general population includes 1% to 5% high myopia, analysis of control 1 may have less power to detect the genetic association with high myopia. Although using emmetropic subjects as

TABLE 5. Genotype Frequency, Associations, and ORs in the High Myopia Patients With CNV and With No CNV

					Genotype Frequency					
SNP	Chr	Position	Genes	Genotype	CNV, %	No CNV, %	Nominal *P**	Adjusted *P*†	Adjusted OR†	95% CI†
rs7837791	8	60179086	*TOX*	GG	22.9	21.6	0.50	0.33	0.92	0.78–1.09
				TG	47.5	52.9				
				TT	29.5	25.5				
rs3138144	12	56114769	*RDH5*	CC	19.7	18.9	0.94	0.80	0.98	0.83–1.16
				CG	49.3	50.6				
				GG	31.1	30.6				
rs8000973	13	100691367	*ZIC2*	CC	11.2	9.7	0.11	0.14	1.14	0.96–1.37
				TC	44.7	41.6				
				TT	44.1	48.7				
rs4778879	15	79372875	*RASGRF1*	AA	17.	18.1	0.60	0.65	0.96	0.81–1.14
				GA	48.5	49.2				
				GG	34.0	32.6				
rs2969180	17	11407901	*SHISA6*	GG	23.2	25.3	0.12	0.04	0.84	0.71–0.99
				AG	47.6	49.8				
				AA	29.2	24.9				

* Differences in the observed genotypic distribution were examined by χ^2 test for trend.
† Age and sex adjustment was performed based on a logistic regression model.

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controls by excluding high myopia will improve the power for detecting a genetic association with high myopia, analysis with control 2 further decreased the significance of the association, partly because of the cohort size. Taken together with its contrasting results relative to those from the original report, we must interpret the association of SNP rs2969180 in the present study with caution.

Genetic factors influencing the risk of developing CNV in myopic eyes have been evaluated in many studies because myopic CNV is the most prominent complication leading to severe visual function loss.[35–39] Genetic variants strongly related to age-related macular degeneration (AMD), another degenerative retinal disease characterized by neovascularization in the macula, have been examined to explain the development of CNV in highly myopic eyes. However, several studies showed that susceptibility genes for AMD did not affect the occurrence of myopic CNV.[35–38] In addition, axial elongation of highly myopic eyes results in the thinning of the retina and choroid, patchy chorioretinal atrophy, and lacquer cracks, all of which are important predisposing conditions for the development of CNV.[12,40,41] Therefore, as another approach, we hypothesized that CNV could occur when the eye is affected strongly by susceptibility genes for myopia. We evaluated the genetic difference between high myopia patients with CNV and those without CNV; however, we found that genotype distribution of the SNPs evaluated did not differ significantly. Among the 5 SNPs, rs2969180 in the *SHISA6* gene showed a *P* value of 0.040, but it was not statistically significant after Bonferroni correction. Because the genetic variants contributing to high myopia and to CNV in high myopic eyes may differ, further analyses are required to assess myopic CNV independent of the susceptibility genes for myopia.

In the current study, we used genotype data in controls that were directly genotyped by arrays to eliminate a possibility of imputation error, which may affect the results. Because two SNPs, rs3138144 in *RDH5* and rs8000973 near *ZIC2*, were not genotyped directly by HumanHap610K Quad Arrays, the number of directly-genotyped control subjects in these two SNPs was smaller than that in the other 3 SNPs.

One of the possible limitations is that the current study may be that it was underpowered for detecting associations with SNPs in *RDH5* (rs3138144) and near *TOX* (rs7837791). A power calculation indicated that to obtain 80% power, we would require odds ratios of >1.22 for SNP rs3138144 and odds ratios of >1.20 for SNP rs7837791 by using the sample size used in the present study. Although we cannot estimate the odds ratios in the case-control study for high myopia, the original report showed that SNPs rs3138144 and rs7837791 had a larger effect on common myopia compared to the other 3 SNPs examined in this study,[32] thereby suggesting that these 2 SNPs required a smaller sample size for their association study. The nonsignificant associations in this study may be caused by other factors, such as heterogeneity across the populations or the discrepancy of responsible genes between common myopia and high myopia. Because the associations between these 2 SNPs and common myopia were replicated successfully in the East Asian population in the original study, these 2 SNPs may explain the difference between the mechanisms involved in the development of common myopia and high myopia. In addition, we examined only the top SNP in each susceptibility locus; therefore, our results do not necessarily negate the associations of the *RDH5* and *TOX* locus to high myopia. To investigate the contribution of these loci to myopia, more detailed, confirmatory studies with larger sample sizes are required.

In conclusion, we showed that genetic variants of SNP rs8000973 near the *ZIC2* gene and rs4778879 in the *RASGRF1* gene are associated with high myopia in Japanese subjects. This result, together with previous GWAS, implied that these SNPs may be the susceptibility loci for myopia and high myopia. However, we were not able to identify genetic factors influencing CNV risk in high myopic patients among these 5 SNPs.

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Appendix

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Vascular Endothelial Growth Factor Gene and the Response to Anti-Vascular Endothelial Growth Factor Treatment for Choroidal Neovascularization in High Myopia

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Purpose: To investigate the association between the *vascular endothelial growth factor (VEGF)* gene polymorphism and the response to anti-VEGF treatment for choroidal neovascularization (CNV) in highly myopic eyes.

Design: Retrospective cohort study.

Participants: A total of 357 unrelated highly myopic Japanese patients with axial lengths ≥26.0 mm in both eyes were eligible, and 83 patients who received anti-VEGF therapy for CNV and could be followed for more than 1 year were included.

Methods: We genotyped a functional single nucleotide polymorphism in the *VEGF* gene, rs2010963. The associations between the distribution of the rs2010963 genotype and the number of eyes with maintained or improved visual acuity (VA) were analyzed. Furthermore, multivariable logistic regression analysis was performed to adjust for 7 possible prognostic factors, including age, sex, CNV size, CNV location, administration of loading dose, pretreatment VA, and number of additional treatments.

Main Outcome Measures: The primary end point was maintenance of VA, and secondary end points were progression of chorioretinal atrophy (CRA) and recurrence of CNV.

Results: Mean age and mean axial length were not significantly different among 3 genotypes of rs2010963. The percentage of eyes with maintained or improved VA was significantly higher with the G allele of rs2010963 ($P = 0.016$), and stepwise analysis revealed that both rs2010963 and CNV size were associated with VA maintenance ($P = 0.040$ and 0.033, respectively). The secondary analysis revealed that administration of a loading dose was significantly associated with both CRA progression ($P = 0.031$) and recurrence of CNV ($P = 0.020$), whereas rs2010963 was not.

Conclusions: These results suggest that the *VEGF* polymorphism influences the VA prognosis in highly myopic eyes with CNV within 1 year after anti-VEGF treatment. This association was still observed after removing its confounding effect through CNV size. The rs2010963 polymorphism was not associated with CNV recurrence or CRA progression, which indicates that these changes are not tied to intrinsic factors and may be controllable by improving treatment methods. *Ophthalmology 2014;121:225-233 © 2014 by the American Academy of Ophthalmology.*

Myopia is the most common visual disorder in the world and a major public health concern, especially in East Asian populations. Its prevalence is estimated to be 25% in the United States and Western Europe and to be higher (40%–70%) in Asians.[1–5] Myopic eyes with very long axial lengths (≥26 mm) or a high degree of myopic refractive error (≤−6 diopters) are classified as high myopia.[6] In highly myopic eyes, choroidal atrophy and choroidal neovascularization (CNV) are the most vision-threatening complications.[7] Choroidal neovascularization mainly affects relatively younger adults aged 40 to 50 years,[8] and its natural history is extremely unfavorable. Visual acuity (VA) at 5 years after the onset of CNV decreases to ≤20/200 in 89% of the eyes and in 96% of the eyes after 10 years.[9] Because it is difficult to prevent the development of myopia and the occurrence of CNV, minimizing VA decline caused by CNV is of great importance for patients with high myopia.

Anti-vascular endothelial growth factor (VEGF) therapy has been used for the treatment of myopic CNV since 2005 and has shown good outcomes.[10–14] Visual acuity improves in many cases, but in some cases anti-VEGF therapy is ineffective. Several studies have been conducted to determine prognostic factors,[12,14,15] and we reported previously that smaller CNV size was a significant prognostic factor predicting better VA.[14]

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Recently, there has been a focus on genetic variants as a prognostic factor for the outcome of CNV treatment in both high myopia and age-related macular degeneration (AMD).[16–18] We reported that the *VEGF* polymorphism is associated with the response to intravitreal bevacizumab (IVB) and triple therapy for CNV secondary to AMD,[17] and that pigment epithelium-derived factor polymorphism is associated with the response of polypoidal choroidal vasculopathy to photodynamic therapy (PDT).[18] In the treatment of CNV secondary to high myopia, although Parmeggiani et al[19] evaluated the association between the genetic variants and the efficacy of PDT, no study has examined the association between the genetic variants and the response to anti-VEGF therapy. Because anti-VEGF therapy is becoming a first choice for treatment of myopic CNV,[20] it is important to evaluate the association between genetic variants and the response to anti-VEGF therapy, which could predict whether anti-VEGF therapy will be effective or not. Recently, we showed that the *VEGF* polymorphism is associated with the size of CNV secondary to high myopia, whereas it is not associated with the occurrence of CNV.[21] In the current study, we evaluated the associations between the *VEGF* polymorphism and the visual outcome after anti-VEGF treatment for CNV in highly myopic eyes.

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

This is a retrospective study that reviewed the medical records of 357 patients with high myopia from whom genomic DNA had been extracted after obtaining informed consent; CNV was found in 158 patients. Patients who underwent anti-VEGF therapy and could be followed for at least 1 year were selected, and a total of 100 eyes were eligible for this study (inclusion/exclusion criteria are discussed later). Because genomic DNA from 17 patients was not available at the time of experiment, 83 patients were finally included in this study. All the patients were recruited from Kyoto University Hospital between September 2005 and April 2011. Each patient underwent a complete ophthalmic examination, including measurement of best-corrected visual acuity (BCVA), indirect ophthalmoscopy and slit-lamp biomicroscopy with a contact lens by a retina specialist, fluorescein angiography (FA) and indocyanine green angiography, and optical coherence tomography (OCT). The BCVA was measured with a Landolt chart and converted to a logarithm of the minimal angle of resolution for statistical analysis.

Inclusion criteria for this study were (1) an axial length of $\geq$26.00 mm or spherical equivalent refractive error of ≤ -6.0 diopters in phakic eyes; (2) fundus changes typical of pathologic myopia, such as chorioretinal atrophy (CRA), lacquer cracks, or atrophic patches; (3) FA documentation of CNV showing active leakage; and (4) follow-up period of at least 12 months after the first anti-VEGF treatment. The exclusion criteria were (1) history of intraocular surgery except for cataract surgery, (2) cataract surgery during the follow-up period, (3) previous anti-VEGF therapy, and (4) other ocular disease that can influence the BCVA, such as corneal opacity or myopic foveoschisis. If patients underwent bilateral anti-VEGF treatment, the eye treated earlier that fulfilled the criteria of this study was selected as the study eye for analysis.

Anti-Vascular Endothelial Growth Factor Therapy

The intravitreal dose of anti-VEGF was as follows: bevacizumab 1.25 mg, pegaptanib 0.3 mg, or ranibizumab 0.5 mg. All injections were performed under sterile conditions, and prophylactic topical antibiotics were applied from a few days before to 1 week after the injection. Anti-VEGF therapy was initiated by single injection or 3 monthly injections. After induction, ophthalmological examinations were performed at scheduled visits of 1-month intervals. Although most patients were examined every month for 1 year, some patients underwent scheduled examinations at 2- to 3-month intervals after resolution of exudative change. Repeat injection was recommended pro re nata on the basis of the judgment of the evaluating clinician. The re-injection criteria included any of the following findings: (1) persistence or recurrence of macular edema or serous retinal detachment in the OCT images, (2) persistence or recurrence of dye leakage in the FA images, (3) new subretinal hemorrhage from the myopic CNV, and (4) worsening of subjective symptoms, such as metamorphopsia, central scotoma, paracentral scotoma, or VA loss.

Genotyping

We genotyped one of the VEGF single nucleotide polymorphisms (SNPs), rs2010963, the C allele that we recently reported to be associated with larger myopic CNV.[21] This SNP has been shown to affect VEGF expression,[22] and its association with various diseases, such as AMD, diabetic retinopathy, Behçet's disease, Alzheimer's disease, and diabetes, has been evaluated.[23–32]

Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L, Fujifilm, Minato, Tokyo, Japan). Rs2010963 was genotyped using a TaqMan SNP assay with the ABI PRISM 7700 system (Applied Biosystems, Foster, CA). Deviations from the Hardy–Weinberg equilibrium (HWE) in genotype distributions were assessed using the HWE exact test.

Outcomes and Statistical Analysis

The primary end point of this study was VA maintenance from the baseline; we examined whether the patient's VA was the same or improved by a Landolt chart at the visit 1 year after the first anti-VEGF treatment. For the secondary end points, we evaluated the recurrence of CNV and CRA progression. The progression of CRA was judged according to our previous report.[33] Briefly, 2 of the authors (A.O. and K.Y.) judged color fundus photographs as a binary trait based on changes in patchy atrophy. When the 2 authors disagreed, a third author (A.T.) was asked to arbitrate. The association of these conditions with the rs2010963 genotype was analyzed using the Cochran–Armitage test.

To specifically evaluate the contribution of the rs2010963 SNPs to the end points, we performed multivariable logistic regression analysis to adjust for age, sex, and previously reported prognostic factors, such as CNV size, pretreatment VA, CNV location, initial loading dose, and number of additional anti-VEGF drug injections after the initial single or 3 monthly injection treatments.[13,14] The location of CNV, subfoveal or not, was treated as a binary trait. After fitting a full model, we selected variables by a stepwise method. When analyzing the association with recurrence, we did not use the number of treatments as a possible predictive factor,

Table 1. Patient Characteristics

Variable	Value
Total (n)	83
Mean age (yrs ± SD)	64.5±11.2
Sex (male:female)	18:65
Mean axial length (mm ± SD)	29.00±1.60
Mean pretreatment BCVA (logMAR)	0.65±0.42
CNV location	
Subfoveal (n, %)	62 (74.7%)
Juxtafoveal (n, %)	18 (21.7%)
Extrafoveal (n, %)	3 (3.6%)
History of other treatment	
None	73
PDT	9
Triamcinolone acetonide	1
Drug	
Bevacizumab	61
Ranibizumab	20
Pegaptanib	2
CNV size (mm^2)	
Mean ± SD	0.93±0.83
Range	0.05–3.43
Greatest linear dimension (mm)	
Mean ± SD	1383±687
Range	390–3370

BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; logMAR = logarithm of the minimum angle of resolution; PDT = photodynamic therapy; SD = standard deviation.

because a high number of treatments was probably a consequence of recurrence, not the cause of recurrence.

The differences in basic demographics among 3 genotype groups were analyzed by appropriate statistical tests using analysis of variance, Fisher exact test, or linear regression analysis. Tukey–Kramer test for post hoc was performed as needed. These analyses were performed using Software R (R Foundation for Statistical Computing, Vienna, Austria) and PLINK version 1.07 (available at: http://pngu.mgh.harvard.edu/w purcell/plink/index, accessed February 18, 2012). A P value < 0.05 was considered statistically significant.

Results

There were 83 patients with CNV in highly myopic eyes who underwent treatment with bevacizumab (62 eyes), pegaptanib (3 eyes), or ranibizumab (18 eyes). All patients were followed for at least 12 months. Anti-VEGF therapy was initiated by a single injection in 65 eyes (initial loading dose [−] group) and by 3 monthly injections in the other 18 eyes (initial loading dose [+] group). Demographic information and characterization of the patients are shown in Table 1. The CNV was subfoveal in 73.5% of patients, and 88.0% of patients were treatment-naïve. During the follow-up period, individuals received a mean of 1.1 additional injections.

Table 2 describes the demographics and clinical phenotypes of the study population according to rs2010963 genotype. The genotype distribution was CC:CG:GG = 16:34:27. The genotype distribution was in HWE (P = 0.403). There were no differences in age, sex, or axial length in the 3 genotype groups. The number of additional anti-VEGF injections within 1 year showed significant differences (P = 0.036) and was significantly higher in patients with the CC genotype compared with those with CG and GG genotypes (P = 0.045 and 0.049, respectively, with Tukey–Kramer multiple comparison). Likewise, the greatest linear dimension and CNV size were relatively larger in patients with the CC genotype (P = 0.10). Although this association was not statistically significant, this trend was compatible with our previous report showing that CNV size in highly myopic eyes is larger in patients with the rs2010963 CC genotype than in those with the CG and GG genotypes.[21]

The VA outcome also showed a similar association to the VEGF genotype. Figure 1 shows VA improvement from the baseline according to rs2010963 genotype. Eyes with the CC genotype had worse VA outcomes than eyes with the GG or CG genotypes. Although retinal exudative change disappeared after anti-VEGF treatment in patients with CC genotype (Fig 2), only half of the patients with the CC genotype of rs2010963 had maintained VA 1 year after the first injection, whereas more than 80% of patients with the CG or GG genotype (Fig 3) maintained VA. The

Table 2. Clinical Phenotypes of Myopic Patients with Choroidal Neovascularization by rs2010963 Genotype

Characteristics	CC	CG	GG	P Value*
No. of patients (n, %)	16 (19.3%)	34 (41.0%)	27 (32.5%)	
Mean age (yrs ± SD)	66.2±11.8	64.8±10.4	61.5±12.0	0.36
Male:female	4:12	6:28	8:19	0.54†
Mean axial length (mm ± SD)	29.42±1.76	29.07±1.29	28.84±1.88	0.53
Subfoveal CNV (n, %)	14 (87.5%)	24 (70.6%)	19 (70.3%)	0.42
No. of treatments (per 12 mos)	2.81	1.91	1.89	0.036
Presence of loading dose (n, %)	5 (31.3%)	8 (23.5%)	4 (14.8%)	0.20
Pretreatment VA (logMAR)	0.62±0.35	0.64±0.47	0.70±0.44	0.80
Greatest linear dimension (mm)	1651±879	1195±529	1498±732	0.063/0.10‡
CNV size (mm^2)	1.26±1.08	0.76±0.78	0.99±0.76	0.15/0.10‡
Maintenance of VA (n, %)	8 (50.0%)	28 (82.3%)	23 (85.2%)	0.016§
Recurrence in 1 yr (n, %)	9 (56.3%)	7 (20.6%)	12 (44.4%)	0.75§
CRA progression in 1 yr (n, %)	10 (62.5%)	12 (35.2%)	14 (51.9%)	0.73§

CNV = choroidal neovascularization; CRA = chorioretinal atrophy; logMAR = logarithm of the minimal angle of resolution; SD = standard deviation; VA = visual acuity.
*Analysis of variance.
†2×3 Fisher exact test.
‡Recessive model.
§Cochran–Armitage test.

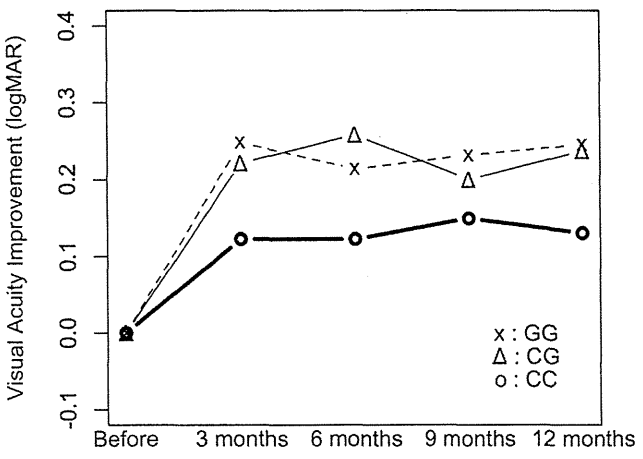

Figure 1. Improvement in visual acuity (VA) according to rs2010963 genotype. Change in VA in logarithm of the minimum angle of resolution from baseline of eyes with the GG, CG, and CC genotypes is plotted at each time point. The VA time course was signi□cantly worse in eyes with the CC genotype than the CG or GG genotype. logMAR = logarithm of the minimum angle of resolution.

statistical analysis revealed a significant association between the rs2010963 genotype and the rate of VA maintenance (Table 2, $P=0.016$). The association was also significant when evaluated with treatment-naïve patients only ($P=0.032$) and when evaluated with only patients with subfoveal CNV ($P=0.041$). As for the secondary end points, recurrence rate and CRA progression within 1 year were not associated with rs2010963 genotype ($P=0.75$ and 0.73, respectively).

The results of the multivariable logistic regression for maintenance of VA are shown in Table 3. Of 8 prognostic factors, the G allele of rs2010963 ($P=0.047$), pretreatment VA ($P=0.025$), and CNV size ($P=0.015$) showed a significant association with the maintenance of VA; odds ratios (ORs) were 2.51, 9.79, and 0.35, respectively. To find the best-fit model, we analyzed the associations with a stepwise method. Vascular endothelial growth factor rs2010963 G allele gave patients a 2.3 times higher tendency to have maintained VA after anti-VEGF treatment ($P=0.040$).

To ensure that the initial treatment method did not affect the genotype–phenotype associations between rs2010963 and VA outcome, we performed 2 analyses. First, we compared the patients' characteristics between the single initial treatment group and the 3 monthly injection group (Table 4). There was no difference in genotype of rs2010963 ($P=0.19$). The VA was maintained in 78.5% of eyes without an initial loading dose and in 77.8% of eyes with an initial loading dose ($P=1.0$). Although patients who underwent an initial loading dose were significantly older than those who did not ($P=0.014$), there were no statistically significant differences in sex ($P=0.22$), axial length ($P=0.19$), pretreatment VA ($P=0.67$), or greatest linear dimension ($P=0.26$). Second, we performed logistic regression analysis using only eyes treated with a single initial treatment (Table 5). This analysis revealed a more significant association of rs2010963 genotype with VA maintenance ($P=0.0080$). In addition to the smaller CNV size ($P=0.0039$), younger age ($P=0.040$) was also a positive predictive factor for VA maintenance.

Finally, we explored factors associated with the recurrence rate of CNV and CRA progression by stepwise analysis (Table 6). The analysis did not select the rs2010963 genotype as a prognostic factor for recurrence and CRA progression within 1 year. Among the 7 factors evaluated, the prognostic factors of recurrence were advanced age (OR, 1.07; $P=0.013$) and presence of loading dose (OR, 0.21; $P=0.031$). The presence of a loading dose also showed a significant association with CRA progression (OR, 4.15; $P=0.020$).

Discussion

In the present study, we demonstrated a significant association between the VEGF rs2010963 genotype and the maintenance of vision after treatment for CNV with anti-VEGF therapy in highly myopic eyes. Parmeggiani et al[19] also evaluated the genetic association for response to myopic CNV treatment. They showed a significant association between 2 genes and CNV responsiveness to PDT. Although the results gave us clinically important information, anti-VEGF therapy is now becoming the first choice for the treatment of CNV in high myopia because of its efficacy and safety.[20] Our findings of the associations between VEGF gene polymorphism and the response to anti-VEGF treatment for CNV in highly myopic eyes in the present study will give us important insight into today's treatment for myopic CNV.

Genetic associations with myopia have been investigated for several decades. Although we reported 2 susceptible loci for high myopia using genome-wide association studies[34,35] and reproduced the association of recently reported myopia susceptibility loci on chromosome 15,[36] susceptibility genes for myopia have not been identified, and it is still difficult to find the means to prevent myopia. Thus, in the clinical setting, controlling not high myopia but its complications is currently the more practical approach. Recently, we reported that the VEGF polymorphism affects the size of myopic CNV, whereas it is not associated with its occurrence. Patients with the C allele of rs2010963 had significantly larger CNV.[21] It has been reported that larger myopic CNV size is a predictive factor for poor outcomes of anti-VEGF treatment.[12–14] Considered together, it would be reasonable to suggest that VEGF polymorphism affects the response to anti-VEGF therapy for CNV in highly myopic eyes. In the current study, however, we showed that both CNV size and VEGF genotype were associated with VA maintenance, even after multivariable logistic regression analysis. The VEGF genotype affected the visual outcome regardless of its effect on the CNV size.

Only 50% of the patients who had the CC genotype of VEGF rs2010963 maintained their VA after anti-VEGF treatment, whereas 80% of the patients with the CG or GG genotype maintained their VA. Because retinal exudative change itself was resolved in all cases after treatment, retinal damage caused by CNV might be different depending on the genotype, and CNV with the C allele might be more harmful to retinal cells. Another possibility is that the potency of the anti-VEGF treatment might be affected depending on the genotype. As shown in Figure 2, some patients still had exudative change 1 week after anti-VEGF treatment, whereas the exudative changes were completely resolved 1 to 3 months after the treatment. Rapid disappearance of retinal exudative change in patients with

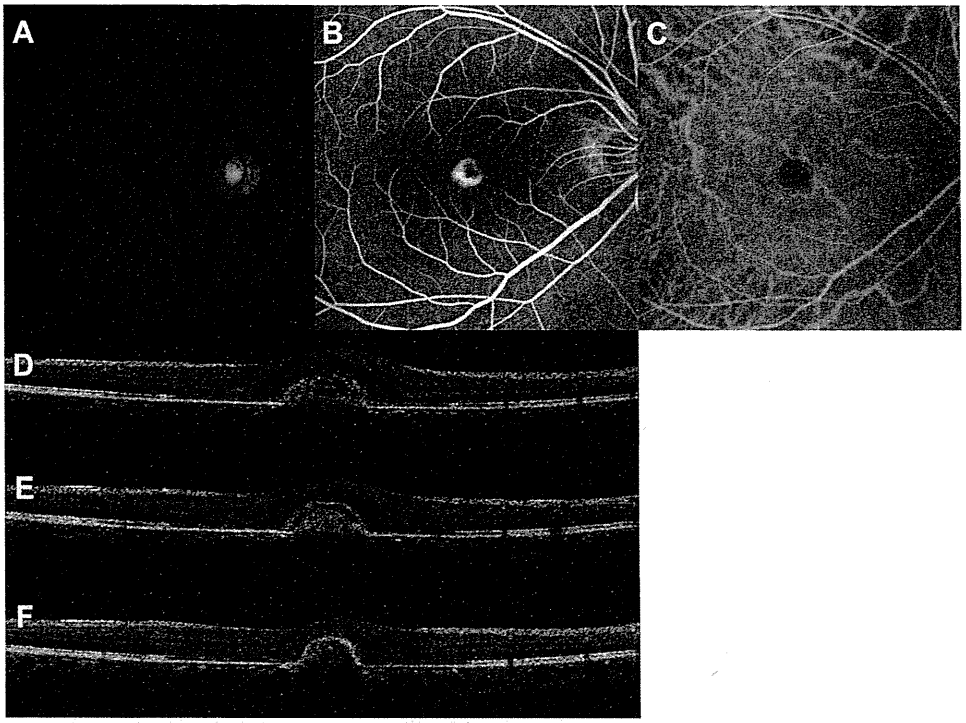

Figure 2. **(A)** Color fundus photograph, **(B)** fluorescein angiography (FA), **(C)** indocyanine green angiograph, and **(D–F)** spectral-domain optical coherence tomography (OCT) images of a patient who received intravitreal bevacizumab (IVB). This 42-year-old woman with choroidal neovascularization (CNV) in her highly myopic right eye had the rs2010963 CC genotype. **(A)** Pretreatment color fundus photograph, **(B)** pretreatment FA, and **(C)** pretreatment indocyanine green angiograph showed CNV beneath the fovea. **(D)** Pretreatment OCT image showed retinal exudative change. **(E)** Exudative change was still present 1 week after IVB and **(F)** almost resolved 1 month after IVB. Decimal VA was improved from 0.4 (pretreatment) to 0.7 (1 week after treatment), 0.7 (3 months after treatment), and 1.2 (1 year after treatment).

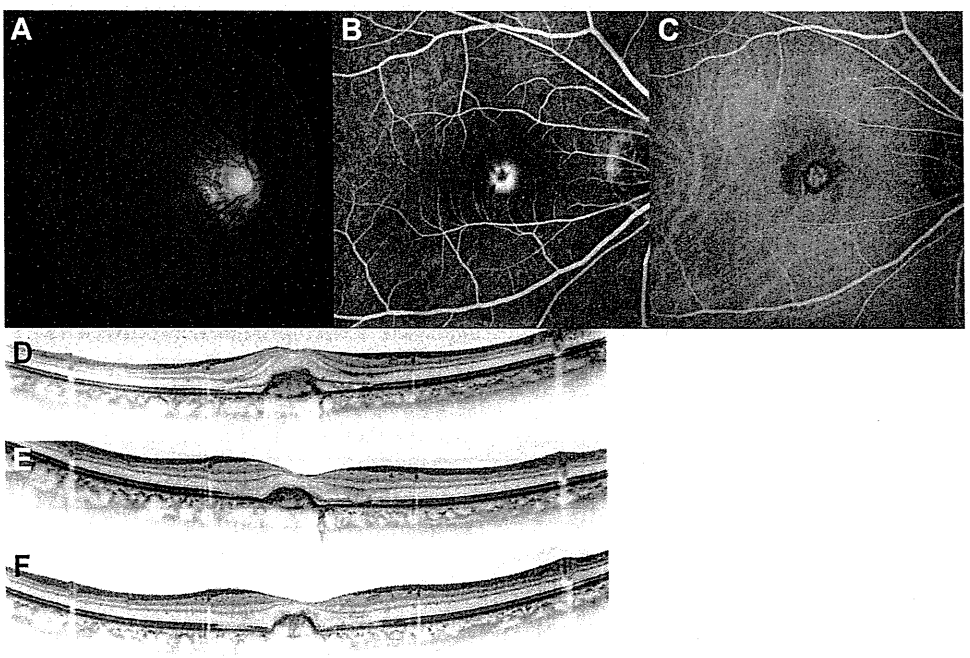

Figure 3. **(A)** Color fundus photograph, **(B)** fluorescein angiography (FA), **(C)** indocyanine green angiograph, and **(D–F)** spectral-domain optical coherence tomography (OCT) images of a patient who received intravitreal bevacizumab (IVB). This 36-year-old man with choroidal neovascularization (CNV) in his highly myopic right eye had the rs2010963 GG genotype. **(A)** Pretreatment color fundus photograph, **(B)** pretreatment FA, and **(C)** pretreatment indocyanine green angiograph showed CNV beneath the fovea. **(D)** Pretreatment OCT image showed retinal exudative change. **(E)** Exudative change almost resolved 1 week after IVB and **(F)** resolved 1 month after IVB. Decimal visual acuity (VA) was improved from 0.4 (pretreatment) to 0.7 (1 week after treatment), 0.8 (3 months after treatment), and 1.5 (1 year after treatment).

Table 3. Multivariable Logistic Regression Analysis for Maintenance of Visual Acuity

	Full Model			Stepwise Method		
	OR	95% CI	P Value	OR	95% CI	P Value
rs2010963 G allele	2.51	1.05–6.64	0.047	2.30	1.06–5.34	0.040
Pretreatment VA (logMAR)	9.79	1.60–93.2	0.025	4.17	0.95–23.8	0.077
CNV size	0.35	0.14–0.78	0.015	0.48	0.23–0.92	0.033
Presence of loading dose	4.15	0.84–28.1	0.10		Not included	
No. of additional treatments	1.38	0.75–2.78	0.33		Not included	
Subfoveal CNV	0.46	0.07–2.28	0.36		Not included	
Age	0.95	0.89–1.01	0.15		Not included	
Sex	0.89	0.19–3.68	0.88		Not included	

CI = con□dence interval; CNV = choroidal neovascularization; logMAR = logarithm of the minimal angle of resolution; OR = odds ratio; VA = visual acuity.

the CG or GG genotype might explain their better visual prognosis. In the current study, however, OCT examination was not performed at 1 week after treatment in all patients. Thus, further evaluation of the remnant exudative change at an early time point might explain the worse visual outcomes in patients with the CC genotype.

Because the mean VA improved even if patients had the CC genotype of rs2010963, highly myopic patients with CNV should be treated with anti-VEGF therapy regardless of their rs2010963 genotype. However, if we examine patients' genotype before treatment, we can predict their visual outcome after anti-VEGF treatment. Furthermore, when new treatments are developed, genotype knowledge would lead to more accurate information of their treatment option.

The need for a loading dose is presently controversial.[37,38] When patients treated with an initial loading dose of 3 monthly injections and patients treated with a single initial injection were evaluated together, our multivariable logistic regression analysis showed that the presence of loading dose was not associated with VA maintenance. Furthermore, the patients' characteristics were not significantly different between patients with a loading dose and patients without a loading dose except for their age. The rs2010963 genotype distribution was not significantly different ($P = 0.19$), and 77.8% of the patients with a loading dose and 78.5% of the patients without a loading dose maintained their VA for 1 year ($P = 1.0$), suggesting that loading dose did not affect our findings of the association between rs2010963 and visual outcome of anti-VEGF treatment for CNV in high myopia. In addition to this analysis, we performed a subanalysis using cases without a loading dose only. This analysis also showed that rs2010963 was significantly associated with the visual outcome ($P = 0.008$). However, both CNV recurrence and CRA progression were associated with the presence of the loading dose. Patients treated with 3 monthly injections showed lower recurrence and more CRA progression when evaluated with stepwise multivariable logistic regression analysis. Although our retrospective study cannot conclude whether treatment method affects recurrence of CNV and CRA progression, considered together with the finding that rs2010963 genotype was not associated with these secondary outcomes, it might be suggested that recurrence of CNV and CRA progression is affected by the method of treatment rather than an intrinsic factor such as genotype.

Uemoto et al[39] have retrospectively reviewed 27 eyes of myopic patients with CNV who underwent IVB treatment and reported that the CNV size, number of treatments, and duration of follow-up were associated with enlargement of CRA. In our study, CNV size was not selected among the factors associated with CRA progression by stepwise analysis, but patients treated with 3 monthly injections showed increased CRA progression (OR, 4.15; 95% confidence interval, 1.31–15.0; $P = 0.020$), and the number of additional

Table 4. Patient Characteristics According to the Presence of Loading Dose

Characteristics	Initial Loading Dose (+)	Initial Loading Dose (−)	P Value
No. of patients	18	65	
Mean age (yrs ± SD)	69.2±7.59	63.2±11.7	0.014
Male:female	2:16	18:47	0.22
Mean axial length (mm ± SD)	29.44±1.56	28.88±1.60	0.19
Pretreatment VA (logMAR)	0.69±0.44	0.64±0.42	0.67
Greatest linear dimension (mm)	1553±708	1336±680	0.26
Maintenance of VA (n, %)	14 (77.8%)	51 (78.5%)	1.00
Rs2010963 genotype (CC:CG:GG)	5:8:4	11:26:23	0.19*

logMAR = logarithm of the minimal angle of resolution; SD = standard deviation; VA = visual acuity.
*Chi-square test for trend.

Table 5. Multivariable Logistic Regression Analysis for Maintenance of Visual Acuity Using Only Eyes Treated with Single Initial Treatment

	Full Model			Stepwise Method		
	OR	*95% CI*	*P Value*	*OR*	*95% CI*	*P Value*
rs2010963 G allele	11.8	2.42–113	0.0093	11.4	2.46–103	0.0080
Pretreatment VA (logMAR)	14.5	1.38–311	0.048	11.8	1.27–210	0.052
CNV size	0.044	0.0032–0.26	0.0048	0.043	0.0032–0.24	0.0039
Presence of loading dose		Not valid			Not valid	
No. of additional treatments	3.71	1.20–20.1	0.064	3.52	1.22–17.9	0.060
Subfoveal CNV	0.80	0.09–6.40	0.83		Not included	
Age	0.90	0.81–0.98	0.034	0.91	0.82–0.99	0.040
Sex	0.49	0.05–3.32	0.49		Not included	

CI = con□dence interval; CNV = choroidal neovascularization; logMAR = logarithm of the minimal angle of resolution; OR = odds ratio; VA = visual acuity.

treatments showed a marginal association (OR, 1.63; 95% confidence interval, 1.00–2.86; $P = 0.062$). More treatment would cause more CRA progression. In contrast, in our previous study that evaluated CRA progression of 22 eyes with myopic CNV for 4 years after IVB, there was not an association between the number of treatments and the CRA progression.[33] Because CRA enlarges in as many as 70% to 80% of patients with myopic CNV when followed for more than 2 years,[13,33] the treatment method would not affect long-term CRA progression.

Study Limitations

The largest limitations are the study's retrospective design and small sample size. However, the patient demographics are similar to previous reports, and it is likely our subjects are representative of the general population with this disease. Furthermore, the results are believable, because most previously reported prognostic factors are found again in our final model that predicts prognosis. Third, we included both treatment-naive patients and patients with previous treatment. Furthermore, we included patients treated with bevacizumab, pegaptanib, or ranibizumab. Although the association of rs2010963 genotype with maintenance of VA also was significant when evaluated with treatment-naïve patients ($P = 0.032$), the influence of such a difference should be further explored. Fourth, we cannot differentiate CRA progression that resulted from CNV regression, high myopia itself, or previous PDT. However, to reduce the influence of CRA due to high myopia, we judged CRA to be progressing only when CRAs were adjacent to the original CNV location. Furthermore, to eliminate the influence of previous PDT, we also conducted the same analysis using treatment-naive patients (Table 7, available at http://aaojournal.org). This analysis still showed a significant association between the CRA progression and the number of additional treatments ($P = 0.043$). Fifth, this is not a comparative study of the untreated patients with CNV. Thus, there remains the possibility that the worse VA outcome is due to its natural course.

In conclusion, we have shown that the VEGF rs2010963 genotype itself is a significant prognostic factor for visual outcome within 1 year after anti-VEGF treatment for CNV in high myopia, even after removing its confounding effect through CNV size. Choroidal neovascularization recurrence and CRA progression were not associated with the rs2010963 genotype but were associated with the mode of treatment. Understanding of both intrinsic and extrinsic factors associated with visual outcomes, CNV recurrence, and CRA progression will help to improve management strategies for high myopia.

Table 6. Stepwise Multivariable Logistic Regression Analysis for Secondary End Points

	Recurrence within 1 Year			CRA Progression after 1 Year		
	OR	*95% CI*	*P Value*	*OR*	*95% CI*	*P Value*
rs2010963 G allele		Not included			Not included	
Pretreatment VA (logMAR)	0.29	0.063–1.16	0.094		Not included	
CNV size	1.98	0.98–4.29	0.065		Not included	
Presence of loading dose	0.21	0.045–0.80	0.031	4.15	1.31–15.0	0.020
No. of additional treatments		Not employed		1.63	1.00–2.86	0.062
Subfoveal CNV	3.57	0.97–15.6	0.068		Not included	
Age	1.07	1.02–1.14	0.013		Not included	
Sex		Not included			Not included	

CNV = choroidal neovascularization; CRA = chorioretinal atrophy; logMAR = logarithm of the minimal angle of resolution; OR = odds ratio; VA = visual acuity.

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Footnotes and Financial Disclosures

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Association Between the Cholesteryl Ester Transfer Protein Gene and Polypoidal Choroidal Vasculopathy

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See the Appendix for the members of the Nagahama Study Group.

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Purpose. To determine whether genetic variants in the lipid-associated genes are related to the risk of developing polypoidal choroidal vasculopathy (PCV) in a Japanese population.

Methods. Five hundred eighty-one patients with PCV and 793 controls were enrolled in the study. Association analysis of allele and genotype frequencies was performed for the following single-nucleotide polymorphisms (SNPs) that are associated with high-density lipoprotein cholesterol levels in blood: rs493258 at the hepatic lipase gene (*LIPC*), rs3764261 at the cholesteryl ester transfer protein gene (*CETP*), and rs12678919 at the lipoprotein lipase gene (*LPL*). A further model adjusting for age-related maculopathy susceptibility 2 (*ARMS2*) A69S, complement factor H (*CFH*) I62V, age, sex, and smoking status was used to confirm the independent association of these SNPs with other covariates.

Results. *CETP* rs3764261 was significantly associated with the development of PCV; the frequency of the minor allele *A* was higher in the PCV cases (24.0%) than in the control subjects (18.5%) ($P = 0.0025$; odds ratio [OR], 1.41; 95% confidence interval, 1.13–1.75). Furthermore, we found an independent association of *CETP* variants with age, sex, smoking status, and genetic background of *ARMS2* A69S, *CFH* I62V, *LIPC* rs493258, and *LPL* rs12678919 ($P = 0.0013$; OR, 1.50). *LIPC* rs493258 and *LPL* rs12678919 did not show significant associations with the development of PCV ($P > 0.05$).

Conclusion. *CETP* variants are associated a risk of developing PCV among the Japanese population.

Keywords: PCV, lipid, *CETP*, case-control study

Polypoidal choroidal vasculopathy (PCV) is characterized by aneurysmal dilations with interconnecting vessels that are best demonstrated by indocyanine green angiography.[1–3] Clinically, PCV is classified into a specific subtype of age-related macular degeneration (AMD), and the incidence of PCV in Asian populations has been reported to be higher than that in Caucasians.[4–6] Controversies exist about the pathogenesis of PCV; whether this condition represents inner choroidal vascular abnormalities or a particular variety of choroidal neovascularization (CNV) remains undetermined. However, because there are apparent differences in the demographic risk profile, clinical course, and visual prognosis, PCV is thought to be a distinct clinical entity.[7] For example, the response to treatment, particularly in photodynamic therapy for PCV, is completely different from that for typical AMD and CNV.[8,9]

Cholesterol and lipids are reported to accumulate underneath the retinal pigment epithelium (RPE) with age. When sufficient debris, including lipids, accumulates and forms a mound between the RPE cell and its basement membrane, it can be seen clinically as drusen. Because many population-based studies have shown the association between drusen and the progression of AMD, drusen is thought to be one of the determinants of both early and late AMD. In fact, an association between high-density lipoprotein (HDL) cholesterol level and the development of AMD has been reported in several studies.[10–12]

Previous studies[13–15] showed that the prevalence of drusen under RPE was reported to be lower in PCV than in AMD. Therefore, the absence of drusen was thought to be one of the criteria necessary to diagnose PCV.[6,15,16] However, the results of a clinical study[16] suggested that drusen is frequently seen in PCV eyes, and several studies[6,17,18] reported that drusen were observed in 20% to 27% of unaffected, fellow eyes in patients with unilateral PCV. Therefore, whether drusen has a functional role in the development of PCV remains controversial.

While previous investigations showed a lower prevalence of drusen among patients with PCV, lipid deposits that distribute from the inner retina to the outer retina are known to be the paramount features of PCV (Figure). Some recent investiga-

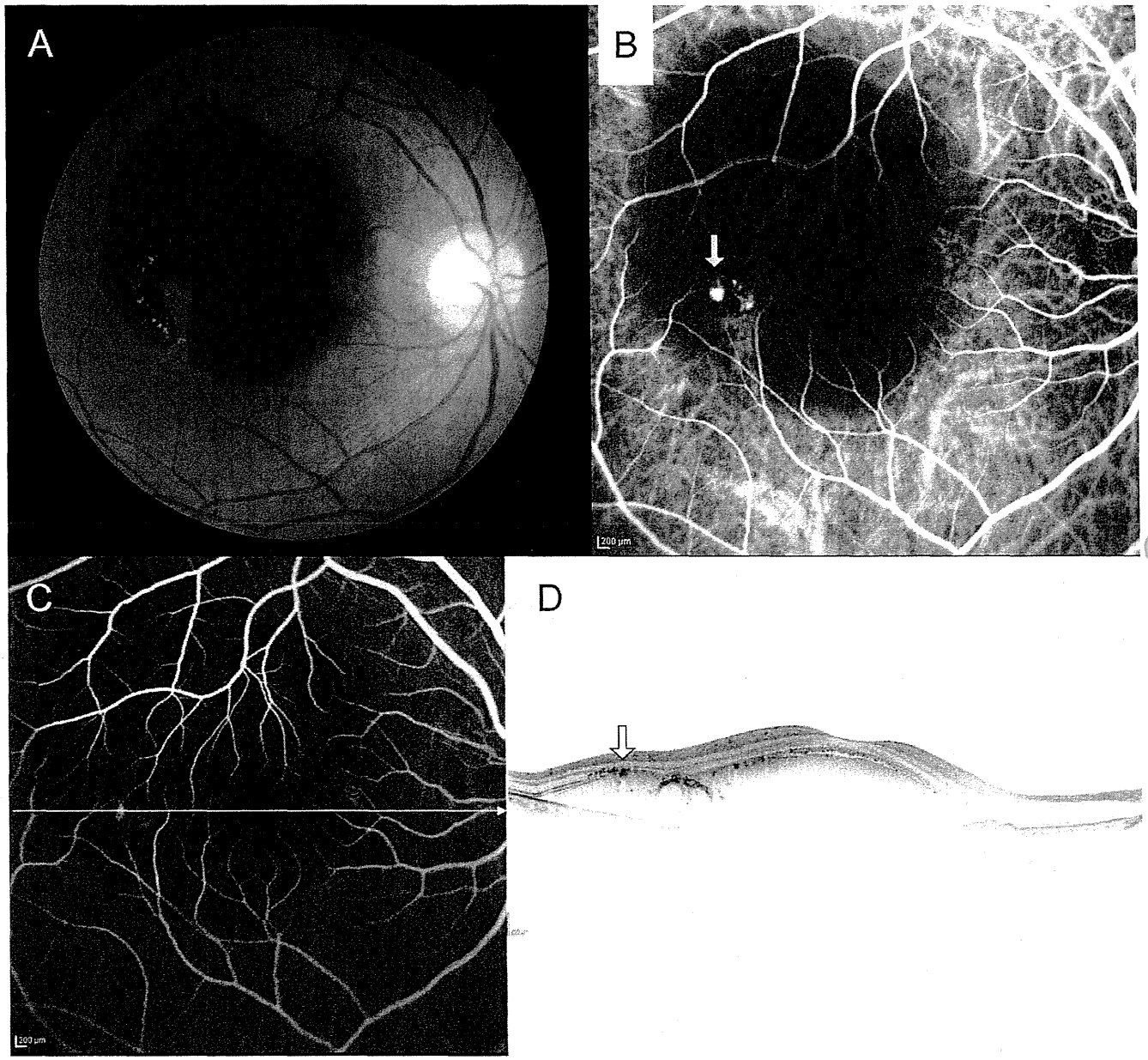

FIGURE. A 64-year-old woman with a typical case of PCV in the right eye. (**A**) Fundoscopic examination shows massive subretinal hemorrhage, lipid deposits, and reddish orange nodules. (**B**) Indocyanine green angiography demonstrates a small branching vascular network terminating in polypoidal lesions (*white arrow*). The speckle noise-reduced spectral-domain optical coherence tomography image of a horizontal section corresponding to the arrow indicated in fluorescein angiography (**C**) shows hyperreflective foci, indicating lipids ([**D**], *arrowhead*), in the outer retina beside the polyp ([**D**], *white arrow*).

tions, including a study[19] in a large cohort of Caucasians, showed significant associations between the lipid-associated genes and the development of AMD. These discoveries of genetic variants in the lipid pathway provided new insight into the pathogenesis of AMD. However, there are limited reports evaluating the association between the lipid-associated genes and the development of PCV. Although several genes are thought to be involved in regulating susceptibility to the development of PCV,[20–23] almost all are identical to those involved in the development of AMD, including the age-related maculopathy susceptibility 2 and high-temperature requirement factor A1 genes (*ARMS2/HTRA1*) locus[24,25] and the complement factor H gene (*CFH*).[26–29] Considering that several studies[13–15] reported a difference in the clinical features of drusen between AMD and PCV, there could be different roles of the lipid-associated genes in these subtypes. Thus, we aimed in this study to determine whether genetic variants in the lipid-associated genes, including variants affecting HDL cholesterol levels, are related to the risk of developing PCV in a Japanese population.

METHODS

All procedures in this study adhered to the tenets of the Declaration of Helsinki, and the ethics committee of each institution involved approved the study protocols. All patients were fully informed about the purpose and procedures of this study, with each patient providing written consent.

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