

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION

Parameters	Patients		Control 1		Control 2	
	High myopia*	Extreme myopia*	Cataract†	P value	Population-based controls	P value
Patients, n	1339	837	334		1194	
Age in years, (mean±SD)	57.2±14.9	57.4±14.1	74.8±8.12	<0.001‡	50.3±15.9	<0.001‡
	Sex, n (%)					
Male	442 (33.0%)	296 (35.1%)	132 (39.5%)	<0.001§	493 (41.3%)	<0.001§
Female	897 (67.0%)	547 (64.9%)	202 (60.5%)		701 (58.7%)	
	Axial length, (mm±SD)					
Right eyes	29.25±1.87	30.18±1.56	22.94±0.88		NA	
Left eyes	29.12±1.83	30.06±1.49	22.97±0.85		NA	
	Refraction of the phakic eyes, D					
Right eyes	-12.83±4.48	-14.41±4.41	-0.459±3.24		NA	
Left eyes	-12.55±4.60	-14.24±4.54	-0.294±2.76		NA	

\* High myopia: axial length  $\geq 26.00$  mm in both eyes. Extreme myopia: axial length  $\geq 28.00$  mm in both eyes. † Individuals who underwent cataract surgery and who had an axial length  $< 25.00$  mm in both eyes. ‡ Unpaired *t* test. Compared with the high myopia group. § Chi-square test. Compared with the high myopia group.

## RESULTS

**Demographics of the study population:** The demographic characteristics of the study population are shown in Table 1. The axial length of the 2,678 eyes of the 1,339 highly myopic cases ranged from 26.00 to 39.73 mm, with a mean  $\pm$  standard deviation (SD) of 29.18 $\pm$ 1.85 mm. Among these 2,678 eyes, 1,881 (70.2%) were phakic. Their mean refraction was  $-12.69\pm 4.54$  D. The axial length of the 668 eyes of the control 1 group ranged from 18.67 to 24.92 mm, with a mean $\pm$ SD of 22.96 $\pm$ 0.87 mm. The mean refraction of the phakic eyes in control 1 was  $-0.379\pm 3.01$  D. The high myopic cases were significantly older and more female-dominant than both control groups ( $p < 0.001$ ).

**Genetic distribution:** The genotype counts and HWE *p* value for the five SNPs in the high myopia, extreme myopia, and control groups are shown in Table 2. Because we calculated the HWE *p* values for 20 genotype distributions, the HWE *p* value cutoff was set to 0.0025, using the Bonferroni correction. Thus, the distributions of the genotypes for the five SNPs were all in HWE.

**Genetic association test:** We evaluated the association between each SNP and high myopia using three models: additive, dominant, and recessive. The *p* values are presented in Table 3. Although no SNP showed a significant association with high or extreme myopia in any models when compared with control 1, the SNP rs5742632 showed a  $p < 0.05$  in the association with extreme myopia when compared with control

2 in the recessive model. However, this SNP did not show any associations after the multiple comparison correction. The other SNPs also showed no association with high and extreme myopia, in any of the models. The lack of association persisted even after an adjustment for age and sex. The adjusted odds ratios are shown in Appendix 1.

We also conducted a haplotype analysis by using a variable-sized sliding-window strategy. This analysis also did not show any significant association. The lowest  $p = 0.147$ , which was observed with a five-SNP window (Table 4). These haplotypes include the rs12423791-rs5742632 haplotype, which has been reported to be associated with extreme myopia.

The statistical power to detect an association of a risk allele with an odds ratio of 1.30 at a significance level of 0.01 is more than 99%. In addition, the statistical power calculation revealed that our sample size detected the gene-disease association for an odds ratio of 1.19 by more than 80%.

## DISCUSSION

Here, we report a case-control study of the association of high and extreme myopia with several polymorphisms, using two Japanese control cohorts. All of the five tagging SNPs of *IGF-1*, including rs6214 and rs12423791, which were suggested to be associated with high or extreme myopia in previous studies, showed no association with high and extreme myopia in the additive, dominant, or recessive model.

TABLE 2. GENOTYPE COUNTS AND HARDY-WEINBERG EQUILIBRIUM P VALUE IN THE HIGH AND EXTREME MYOPIA CASES AND CONTROLS

SNP name	Allele definition		High Myopia					Control 1				
	Allele 1	Allele 2	1/1*	1/2*	2/2*	Allele1 frequency	HWE p value	1/1*	1/2*	2/2*	Allele1 frequency	HWE p value
rs6214	C	T	277	641	373	0.463	0.955	83	159	88	0.492	0.51
rs978458	T	C	256	661	361	0.459	0.144	68	154	110	0.437	0.316
rs5742632	G	A	209	657	410	0.421	0.051	58	151	120	0.406	0.423
rs12423791	C	G	97	452	672	0.265	0.091	23	109	194	0.238	0.169
rs2162679	C	T	178	540	569	0.348	0.007	40	146	145	0.341	0.715

  

SNP name	Allele definition		Extreme myopia					Control 2				
	Allele 1	Allele 2	1/1*	1/2*	2/2*	Allele1 frequency	HWE p value	1/1*	1/2*	2/2*	Allele1 frequency	HWE p value
rs6214	C	T	179	392	223	0.472	0.776	268	585	341	0.469	0.562
rs978458	T	C	158	401	228	0.456	0.473	264	596	334	0.471	1
rs5742632	G	A	123	410	256	0.416	0.057	229	586	379	0.437	0.953
rs12423791	C	G	54	268	421	0.253	0.208	85	468	641	0.267	1
rs2162679	C	T	108	331	352	0.346	0.034	149	541	504	0.849	0.351

\* 1/1: genotype with homozygous allele 1; 1/2: genotype with heterozygous alleles; 2/2: genotype with homozygous allele 2.

*IGF-1* was identified as a high myopia-related protein in two animal model studies. These studies showed that IGF-1 or insulin injection into chick eyes accelerated their axial elongation, whereas glucagon injection decelerated it [24,25]. Penha et al. [26] indirectly supported this in 2011; they reported that hyperopic defocus, a cause of axial elongation of chick eyes, is associated with overexpression of the IGF-1 receptor in chick eyes. In humans, although a significant

association of the *IGF-1* SNP with any myopia and with high myopia was reported in Caucasians in 2010 [16], the association was not confirmed in a Polish family cohort, using the single-SNP association, family-based association, and pedigree disequilibrium tests [17]. Furthermore, two subsequent Chinese studies showed a significant association of *IGF-1* tag SNPs with high or extreme myopia [18,19]. However, these replication studies have limitations due to the evaluation of a

TABLE 3. GENETIC ASSOCIATION TEST FOR 5 SNPs (VERSUS HIGH MYOPIA/EXTREME MYOPIA)

SNP name	Additive model	Additive model	Dominant model	Recessive Model
	Nominal p value*	Adjusted p value†	Nominal p value‡	Nominal p value‡
<b>Control 1</b>				
rs6214	0.175/0.389	0.294/0.456	0.424/0.637	0.150/0.341
rs978458	0.302/0.413	0.586/0.569	0.081/0.162	0.855/0.867
rs5742632	0.466/0.654	0.674/0.822	0.135/0.189	0.587/0.394
rs12423791	0.177/0.453	0.213/0.315	0.148/0.373	0.594/0.906
rs2162679	0.754/0.840	0.568/0.429	0.895/0.845	0.407/0.483
<b>Control 2</b>				
rs6214	0.642/0.857	0.745/0.889	0.855/0.805	0.552/0.971
rs978458	0.402/0.348	0.500/0.362	0.880/0.642	0.205/0.273
rs5742632	0.251/0.177	0.311/0.192	0.836/0.757	0.069/0.039
rs12423791	0.838/0.346	0.805/0.335	0.505/0.212	0.442/0.908
rs2162679	0.815/0.732	0.603/0.572	0.315/0.325	0.320/0.452

\* Trend  $\chi^2$  test. † Generalized linear model. Adjusted by age and sex. ‡ Generalized linear model.

TABLE 4. VARIABLE-SIZED SLIDING-WINDOW HAPLOTYPE ANALYSIS.

Haplotypes	versus high myopia	versus extreme myopia
	lowest p value	lowest p value
<b>2-SNP window</b>		
rs6214-rs978458	0.425	0.497
rs978458-rs5742632	0.572	0.422
rs5742632-rs12423791	0.712	0.447
rs12423179-rs2162679	0.149	0.493
<b>3-SNP window</b>		
rs6214-rs5724632	0.246	0.53
rs978458-rs12423791	0.564	0.336
rs5742632-rs2162679	0.252	0.327
<b>4-SNP window</b>		
rs6214-rs12423791	0.298	0.306
rs978458-rs2162679	0.293	0.343
<b>5-SNP window</b>		
rs6214-rs2162679	0.147	0.373

small cohort in each study (127, 300, and 302 cases, respectively). Small cohorts do not faithfully represent the larger population, which can lead to the generation of false-positive results. In addition, an analysis of a small cohort only has low statistical power, which can also give rise to false-negative results.

We conducted a systematic case-control study to evaluate the association of *IGF-1* with high myopia in the Japanese population, using a relatively large cohort of 1,338 high myopic cases, 334 cataract controls, and 1,194 healthy Japanese controls; the statistical power of the single-SNP analysis was quite high ( $\geq 99\%$ ). Despite the high statistical power, all of the five tagging SNPs selected to cover 100% of the major *IGF-1* SNPs showed no association with high and extreme myopia, though we fit all the hereditary models. Furthermore, haplotype analysis with the powerful variable-sized sliding-window strategy did not reveal any significant association with high and extreme myopia. The inclusion criteria for the present study are more suitable than those of the previous studies; we used axial length as an indicator of myopia, whereas the previous studies (except Zhuang's study [19]) used spherical equivalent. In the chick study, insulin or IGF-1 injection into chick eyes accelerated their axial elongation [24,25], implicating both as possible causes of axial myopia, rather than refractive myopia. Given that the previous studies included refractive myopia cases, of which only a proportion are axial myopia, we infer that they lack the power to rigorously test the association between *IGF-1* SNPs and axial myopia.

At first glance, this result seems to conflict with the previously mentioned chick study [24,25,29]. However, this may be due to differences between avians and mammals. First, chick eyes have cartilage in the sclera, whereas mammalian eyes do not [30]. This structural difference may affect the response to IGF-1 because this hormone is also proposed to affect the anabolism of cartilage matrix molecules [31]. Second, the biochemistry and signaling cascades from the retina to the sclera differ between chicks and mammals. For instance, glucagon, which plays an important role in inhibiting myopia in the chick model, had no effect in a mouse model [29,32]. Similarly, the all-trans-retinoic acid levels in the retinal pigment epithelium during induction of myopia were inversely proportional in chick and marmosets [33,34]. Therefore, it is possible that IGF-1 has an effect on the development of myopia in birds but not in humans.

Our study does have some limitations. The first is the SNP selection. Although the five evaluated SNPs covered 100% of all the major *IGF-1* SNPs, we targeted only major SNPs. Relatively minor and untaggable SNPs might be functionally important in the onset or pathological mechanism of high myopia. However, although the original study [16] evaluated a total of 13 tagging SNPs that showed a MAF  $\geq 5\%$  in the *IGF-1* gene, the study reported that only rs6214, whose MAF is 42%, showed a significant association with high myopia. Furthermore, the subsequent Chinese studies evaluated tagging SNPs that showed MAFs  $\geq 10\%$  in the *IGF-1* gene, and only rs12423795, whose MAF is 31%, showed a significant association [18,19]. Hence, it seems futile to

evaluate minor SNPs when testing the association of *IGF-1* SNPs with high myopia. Second, we did not trace the method of Mak's study, which showed a significant association of *IGF-1* haplotypes with refractive high myopia. Accordingly, we cannot negate the association, in the strict sense. However, our present study, which used the variable sliding-window haplotype analysis (Table 4), of a large cohort has clear and strong implications. Third is the possibility that some individuals in the control 2 group (general population control) might have or develop high myopia. This may decrease the statistical power to some extent. However, since the prevalence of high myopia in Asians is reported to be 1% to 10% [35,36], the loss of the statistical power must be limited. Finally, the geographical difference of control 2 may induce potential sampling biases. However, because the Japanese population has been reported to have a rather small genetic diversity, according to data from the SNP discovery project in Japan [37], the influence of the geographical difference would be small.

In summary, we showed that none of the major tagging SNPs of *IGF-1* were associated with high and extreme myopia in a large cohort of Japanese subjects. These findings do not support the positive results of previous studies that evaluated various ethnicities. More work is required to determine the involvement of the *IGF-1* gene.

#### ACKNOWLEDGMENTS

Supported, in part, by grants-in-aid for scientific research (Nos. 21249084 and 22791653) from the Japan Society for the Promotion of Science, Tokyo, Japan. The funding organization had no role in the design or conduct of this research.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 21 May 2013. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

# Evaluation of Pigment Epithelium-Derived Factor and Complement Factor I Polymorphisms as a Cause of Choroidal Neovascularization in Highly Myopic Eyes

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Submitted: April 23, 2013

Accepted: May 22, 2013

Citation: Miyake M, Yamashiro K, Nakanishi H, et al. Evaluation of pigment epithelium-derived factor and complement factor I polymorphisms as a cause of choroidal neovascularization in highly myopic eyes. *Invest Ophthalmol Vis Sci*. 2013;54:4208-4212. DOI:10.1167/iov.13-12280

**PURPOSE.** A case-control study in a relatively large cohort of highly myopic patients was conducted to explore the genetic background of the occurrence of choroidal neovascularization (CNV) secondary to high myopia.

**METHODS.** We evaluated three single nucleotide polymorphisms (SNPs) from two candidate genes: pigment epithelium-derived factor (*PEDF*) and complement factor I (*CFI*). The SNPs were selected based on previous reports. A total of 1082 unrelated highly myopic (i.e., axial length  $\geq 26$  mm in at least one eye) Japanese individuals with CNV ( $n = 478$ ) and without CNV ( $n = 557$ ) who were 50 years of age and older were genotyped by using an SNP assay. Multivariable logistic regression was conducted to adjust for age, sex, and axial length.

**RESULTS.** Compared with individuals without CNV, subjects with CNV were significantly older ( $P < 0.01$ ) and more likely to be female ( $P < 0.01$ ), but they did not have a significantly different axial length ( $P = 0.50$ ). We did not find an association between the three SNPs and the occurrence of CNV. However, a subanalysis using extremely myopic patients (case:control = 284:317) revealed a marginal association of rs12603825 in the *PEDF* gene ( $P = 0.045$ ). The contribution of rs1136287 in *CFI* was not found in any analysis.

**CONCLUSIONS.** We demonstrated a marginal association of the *PEDF* SNP, rs12603825, with myopic CNV in extremely myopic patients. A further study using a larger cohort might elucidate a significant association; rs1136287 in *CFI* is less likely to be associated in Japanese individuals.

**Keywords:** high myopia, choroidal neovascularization, genetics, *PEDF*, *CFI*

Myopia is one of the most common ocular disorders worldwide. Its prevalence in the United States and Western Europe is estimated to be 25%, and the condition is far more prevalent in Asia (40%-70%).<sup>1-5</sup> Eyes with very long axial lengths ( $\geq 26$  mm) or a high degree of myopic refractive error ( $\leq -6$  diopters [D]) are diagnosed as high myopia,<sup>6</sup> which is one of the major causes of legal blindness in developed countries.<sup>7-9</sup> Highly myopic eyes are often affected by a variety of myopic complications.<sup>10</sup> Among them, choroidal neovascularization (CNV), secondary to high myopia, is a severe health concern because it usually affects adults in the fourth and fifth decades of life, leading to an extremely poor visual prognosis: the visual acuity at 5 and 10 years after the onset of CNV decreased to  $\leq 20/200$  in 89% and in 96% of eyes, respectively.<sup>11,12</sup> Because preventing myopia itself is presently difficult, it is of great importance to investigate the mechanisms of CNV occurrence and growth in highly myopic eyes.

Although a wealth of evidence has shown that the occurrence of CNV observed in age-related macular degeneration (AMD) is associated with the patient's genetic background,<sup>13-19</sup> only limited studies have explored the genetic background of the occurrence of CNV secondary to high

myopia. Fernandez-Robredo et al.,<sup>20</sup> who first evaluated the genetic background of myopic CNV, failed to show an association with established disease-susceptible genes of AMD, age-related maculopathy susceptibility 2 (*ARMS2*) and complement factor H (*CFH*). Thereafter, we conducted three studies to investigate the genetic background of myopic CNV by evaluating likely candidate genes (or loci) such as *ARMS2*, *CFH*, *HTRA* serine peptidase 1 (*HTRA1*), 15q14, 15q25, and vascular endothelial growth factor A (*VEGFA*), but we did not find any susceptible genes.<sup>21-23</sup> However, *VEGFA* showed a significant association with the size of myopic CNV, although it did not show an association with the occurrence of myopic CNV.<sup>23</sup> In addition, a recent study reported a positive association between the complement factor I (*CFI*) gene polymorphism, rs10033900, and the occurrence of CNV by using 71 cases and 196 controls in Caucasians.<sup>24</sup> These results indicate that genetic background plays a role in CNV observed in AMD and is also secondary to high myopia.

Serpin peptidase inhibitor, clade F (*SERPINF1*), also known as pigment epithelium-derived factor (*PEDF*), is a major protein that affects angiogenesis to the same extent as *VEGF*; however, in contrast to the angiogenic effect of *VEGF*, *PEDF* has

an antiangiogenic effect.<sup>25-27</sup> Several groups have evaluated *PEDF* as a candidate gene for neovascular diseases such as diabetic retinopathy and AMD.<sup>28-34</sup> Regarding CNV, Lin et al.<sup>29</sup> reported a positive association between a single nucleotide polymorphism (SNP), rs1136289, and AMD. Although the association of this SNP has not been replicated to date,<sup>30,32,34</sup> our group showed that another SNP in *PEDF*, rs12603825, was associated with the response of polypoidal choroidal vasculopathy to photodynamic therapy (PDT).<sup>33</sup> Taken together, these findings indicate that *PEDF* is a possible candidate gene that may be responsible for the occurrence of CNV secondary to high myopia and is worth being evaluated further.

In the current study, we evaluated three SNPs from *CFI* and *PEDF* as disease-susceptible polymorphisms for myopic CNV (mCNV) by using a large, highly myopic cohort consisting of 478 cases and 557 controls.

## METHODS

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

### Patients and Controls

We recruited 478 unrelated highly myopic Japanese patients with CNV who were  $\geq 50$  years of age (mean age  $\pm$  SD, 66.7  $\pm$  8.6 years; male:female, 87:391) from Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. The inclusion criteria were (1) high myopia (axial lengths  $\geq 26.00$  mm) in at least one eye, (2) clinical presentation and angiographic manifestations of macular CNV in at least one highly myopic eye, and (3) age  $\geq 50$  years at the first visit with CNV to our institutes. All of the patients underwent detailed ophthalmologic examinations, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, measurement of the axial length by A-scan ultrasound (UD-6000; Tomey, Nagoya, Japan), or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA), color fundus photography, optical coherence tomography, and fluorescein angiography. Individuals with a history of ocular surgery, with the exception of cataract surgery, were excluded. Patients with secondary choroidal neovascular diseases, such as angioid streaks, presumed ocular histoplasmosis syndrome, and ocular trauma, were also excluded. When the patient had CNV in both eyes, we used the length of the eye with the longer axial length for the statistical analysis.

As control subjects, 557 highly myopic (axial lengths  $\geq 26.00$  mm in at least one eye) Japanese individuals who were 50 years of age and older (64.3  $\pm$  8.9 years; male:female, 187:370) without CNV were recruited from Kyoto University Hospital, Tokyo Medical and Dental University Hospital, and Ozaki Eye Hospital. We used the length of the eye with the longer axial length for statistical analysis.

For the subanalysis, we evaluated the association of three SNPs with mCNV in extreme myopia patients. The inclusion criteria for the extreme myopia group were (1) the presence of extreme myopia (axial lengths  $\geq 28.00$  mm) in at least one eye, (2) clinical presentation and angiographic manifestations of macular CNV in at least one extremely myopic eye, and (3) 50 years of age and older at the first visit with CNV to our institutes. The inclusion criteria for the control group were (1)

extreme myopia (axial lengths  $\geq 28.00$  mm) in at least one eye, (2) no clinical presentation of macular CNV in either eye, and (3) 50 years of age and older at the first visit to our institutes. To evaluate cases that were more extreme, the criteria of axial lengths  $\geq 29.00$  mm and  $\geq 30.00$  mm were also applied for further analysis.

## Genotyping

Genomic DNAs were prepared from peripheral blood by using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). *PEDF* polymorphisms rs1136287 and rs12603825, which are the only SNPs in *PEDF* previously reported to be associated with CNV observed in AMD,<sup>29,33</sup> were genotyped in all patients by using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). We also genotyped the *CFI* polymorphism rs10033900, which is the only SNP previously reported to be associated with CNV secondary to high myopia.<sup>24</sup>

## Statistical Analyses

The differences in age, axial length, and the spherical equivalent (SE) of the two groups were compared by using the unpaired *t*-test and the difference in sex was compared by using the Fisher's exact test. Deviations from the Hardy-Weinberg equilibrium (HWE) in genotype distributions were assessed for each group by using the HWE exact test. The Cochran-Armitage test was used to compare the genotype distributions of the two groups. Multiple regression and logistic regression analysis were performed to adjust for age, sex, and axial length.

All statistical analyses were conducted by using R Software (R Foundation for Statistical Computing, Vienna, Austria; available in the public domain at <http://www.r-project.org/>) and PLINK (ver. 1.07; available in the public domain at <http://pngu.mgh.harvard.edu/~purcell/plink/index>). A value of  $P \leq 0.05$  was considered statistically significant. The Bonferroni correction was used for multiple comparisons.

## RESULTS

The demographics of the participants are shown in Table 1. Of the total of 1082 patients that were included in this study, 478 patients (44.2%) had CNV and 557 patients (51.5%) did not. Patients with CNV were significantly older and more likely to be female ( $P < 0.001$  for both), whereas no significant differences were found in axial length and SE ( $P = 0.50$  and  $0.36$ , respectively). The mean axial length and SE of all patients were  $29.49 \pm 1.84$  mm and  $-13.40 \pm 4.69$  D, respectively.

The genotype counts, associations, and odds ratios (ORs) for the three SNPs in the highly myopic patients with and without CNV are shown in Table 2. The genotype distributions of the three SNPs were in HWE ( $P > 0.05$ ). This analysis did not reveal any significant association with the occurrence of mCNV ( $P = 0.35$ ,  $0.32$ , and  $0.86$ ), even after adjustment for age, sex, and axial length ( $P = 0.43$ ,  $0.36$ , and  $0.66$ , respectively).

The results from the subsequent analysis on extreme myopia patients are shown in Table 3. As described in the Methods section, we used three definitions for extreme myopia: (1) axial length  $\geq 28.00$  mm in at least one eye, (2) axial length  $\geq 29.00$  mm in at least one eye, and (3) axial length  $\geq 30.00$  mm in at least one eye, which resulted in the inclusion of 843, 629, and 393 patients, respectively. After adjusting for age, sex, and axial length, rs1136287 and



TABLE 1. Characteristics of the Study Population

Population Characteristics	Total*	CNV (+)	CNV (-)	P Value†
Patients, n (%)	1082	478 (44.2%)	557 (51.5%)	—
Age, y; mean ± SD	65.5 ± 8.7	66.7 ± 8.6	64.3 ± 8.9	<0.001
Sex, male:female	287:795	87:391	187:370	<0.001‡
Axial length, mean ± SD	29.49 ± 1.84	29.47 ± 1.68	29.55 ± 1.96	0.50
Refraction of the phakic eye, mean ± SD	-13.40 ± 4.69	-13.60 ± 4.75	-13.26 ± 4.61	0.36

\* Patients who had high myopia (axial length ≥ 26 mm) in at least one eye and were ≥50 years of age were recruited.

† Unpaired *t*-test.

‡ Fisher's exact test.

rs10033900 did not show an association with any definition of extreme myopia, whereas rs12603825 showed a significant association ( $P = 0.045$ ) with an OR of 1.30 (95% confidence interval [CI], 1.00-1.69) when evaluated based on definition 2. However, this association was no longer significant after multiple comparison correction. rs1136287 in *CFI* did not show a significant association in any analysis.

DISCUSSION

In the current study, we demonstrated a possible association between the *PEDF* SNP, rs12603825, and the occurrence of CNV in extreme myopia patients (defined by an axial length ≥ 29.00 mm at least one eye) with an OR of 1.30 ( $P = 0.045$ ). Although we cannot emphasize this result because it was not significant after multiple testing, we believe it has potential importance in the investigation of so-called myopic CNV. On the other hand, the association of the *CFI* polymorphism rs10033900 was not replicated in this study.

Although the genetic background of CNV observed in AMD has been evaluated by many groups, that of CNV secondary to high myopia has not been fully evaluated. Fernandez-Robredo et al.<sup>20</sup> and our group showed no association between the occurrence of CNV secondary to high myopia and *ARMS2* or *CFH*.<sup>21</sup> We also found no association from the evaluation of *VEGFA*, 15q14, and 15q25.<sup>22,23</sup> Recently, Leveziel et al.<sup>24</sup> evaluated 15 genes that were reported to be related to AMD, and showed that only one SNP within the *CFI* gene was associated with the occurrence of CNV secondary to high myopia. However, *PEDF* which was also reported to be related to AMD,<sup>29</sup> was not included in their study. Considering its antiangiogenic effect, *PEDF* warrants evaluation.

In the present study, we selected two SNPs in the *PEDF* gene and one SNP in *CFI* for evaluation. rs1136287 in *PEDF*

was reportedly associated with AMD in a Taiwanese cohort, but this finding was negated by subsequent studies.<sup>29</sup> On the other hand, we previously reported that rs12603825 in *PEDF* is associated with the response of AMD to PDT.<sup>33</sup> Because other SNPs within the *PEDF* gene have never been reported to be associated with CNV,<sup>30,32,34</sup> these two SNPs were appropriate for the first evaluation of the *PEDF* gene. Simultaneously, we attempted to replicate the association between *CFI* and the occurrence of mCNV. Allele frequency was almost consistent with 1000 genomes JPT (Japanese in Tokyo) data.

A marginal association with rs12603825 was seen only in a subset of patients with extreme myopia, as defined by an axial length ≥ 29.00 mm in at least one eye. This result is not surprising because refining the phenotype of the study population, which usually enhances the statistical power if the number of the study population, is adequate. By using the same logic, we recruited only patients 50 years of age and older. The result of the current study shows that the risk of myopic CNV occurrence increases with an odds ratio of 1.30 when patients have an A allele of rs12603825. Because our previous report showed that an A allele of rs12603825 was associated with a poor response of AMD to PDT,<sup>33</sup> the A allele of this SNP may weaken the antiangiogenic effect of the *PEDF* gene. The function of this SNP should be explored further.

On the other hand, we failed to replicate the contribution of the *CFI* polymorphism to myopic CNV. For rs10033900, in which the minor allele frequency (MAF) is 0.35, the statistical power calculation revealed that our sample size could detect the gene-disease association for an odds ratio of 1.44 by more than 80%. Assuming ORs are to be 1.91 as reported by previous report,<sup>24</sup> this study could detect the association by 99.9%. Thus, rs10033900 in *CFI* is less likely to be associated with mCNV in Japanese individuals. Although our study showed same MAF of this SNP between cases, controls, and even 1000 genomes JPT data, Leveziel et al.<sup>24</sup> showed disparity in T allele

TABLE 2. Genotype Counts, Associations, and Odds Ratios in the Highly Myopic Patients With and Without CNV

Gene	SNP	Genotype	CNV (+)		CNV (-)		1000 Genome JPT	Statistical Analysis		
			n	MAF	n	MAF	MAF	Nominal P*	Adjusted P†	Adjusted OR (95% CI)
<i>PEDF</i>	rs12603825	GG	234	0.292	288	0.273	0.287	0.35	0.43	1.08 (0.89-1.32)
		GA	192		219					
		AA	40		40					
<i>PEDF</i>	rs1136287	CC	130	0.483	161	0.461	0.494	0.32	0.36	1.09 (0.91-1.30)
		CT	234		277					
		TT	114		118					
<i>CFI</i>	rs10033900	TT	200	0.357	225	0.360	0.354	0.86	0.66	1.04 (0.87-1.25)
		TC	197		233					
		CC	67		76					

CI, confidence interval.

\* Cochran-Armitage test.

† Logistic regression analysis. Adjusted for age, sex, and axial length.

TABLE 3. Genotype Counts, Associations, and Odds Ratios in Extreme Myopia Patients With and Without CNV

Gene	SNP	Allele		Axial Length, mm	CNV (+)			CNV (-)			Statistical Analysis*	
		1	2		1/1	1/2	2/2	1/1	1/2	2/2	Adjusted P Value	Adjusted OR (95% CI)
PEDF	rs12603825	G	A	≥28	186	158	36	219	170	27	0.099	1.20 (0.96-1.51)
				≥29	122	128	27	164	131	22	0.045	1.30 (1.00-1.69)
				≥30	69	71	16	113	86	16	0.088	1.32 (0.95-1.84)
PEDF	rs1136287	C	T	≥28	106	185	98	117	224	81	0.14	1.16 (0.95-1.42)
				≥29	71	142	71	85	175	62	0.16	1.18 (0.93-1.50)
				≥30	44	78	39	58	123	39	0.37	1.14 (0.84-1.55)
CFI	rs10033900	T	C	≥28	160	160	55	169	186	54	0.63	1.05 (0.85-1.29)
				≥29	115	118	42	130	143	40	0.49	1.09 (0.85-1.38)
				≥30	68	67	23	85	104	26	0.96	1.01 (0.74-1.36)

\* Logistic regression analysis. Adjusted for age, sex, and axial length.

frequency between cases (0.51), controls (0.35), total (0.39), and 1000 genomes CEU data (0.45), suggesting that this SNP might be associated with not only CNV in high myopia but also high myopia itself in Caucasians. This issue needs to be explored in a large Caucasian cohort in the future.

This study had several strengths and limitations. The strength of this study was its large sample size, wherein we evaluated a total of 1082 highly myopic patients that included 478 individuals with CNV. In contrast, Levezief et al.<sup>24</sup> treated only 267 highly myopic patients that included 71 individuals with CNV. It is known that a large cohort increases the statistical power and is more likely to represent the population, which reduces both false negatives and false positives. The second strength is that the phenotype of our cohort was well refined. For example, we recruited only individuals 50 years of age and older to avoid the risk that the control group would develop CNV in the future. In addition, the mean axial length and SE were not significantly different in the two groups. This homogeneity contributes to canceling the "noise" of genetic background, which cannot be eliminated by statistical adjustment. However, the sample size of this study was also a limitation. For rs12603825, in which the MAF is 0.26 in HapMap II JPT, in general, the statistical power to detect an association of a risk allele with an odds ratio of 1.50 is 83.6% when using 500 cases and 500 controls, and is 62.3% when using 300 cases and 300 controls. In the current study design, the significance level is 0.0166 after Bonferroni correction. To achieve this significance level by a statistical power of 80%, we need 607 cases and 607 controls. Thus, whereas the design of the current study that used highly myopic patients was appropriate, that of the subset analysis using extremely myopic patients may not have been appropriate. A larger cohort of extremely myopic patients should be evaluated because a marginal association was found in the current study.

In summary, we demonstrated a possible association between the PEDF SNP rs12603825 and the occurrence of myopic CNV in extremely myopic patients ( $P \leq 0.05$ ), but we did not find an association for the CFI rs10033900. Although we cannot put too much emphasis on the association of PEDF because of its effect size and the lack of significance after multiple comparisons, this result is important regarding the investigation of the cause of myopic CNV. Since our study lacks functional data regarding to rs12603825, further replication of the association and supportive functional data are needed.

Acknowledgments

Supported in part by Grants-in-Aid for Scientific Research 21249084 and 22791653 from the Japan Society for the Promotion of Science, Tokyo, Japan. The authors alone are responsible for the content and writing of the paper.

Disclosure: M. Miyake, None; K. Yamashiro, None; H. Nakanishi, None; I. Nakata, None; Y. Akagi-Kurashige, None; K. Kumagai, None; M. Oishi, None; A. Tsujikawa, None; M. Moriyama, None; K. Ohno-Matsui, None; M. Mochizuki, None; N. Yoshimura, None

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