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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M yopia 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%).1–5 Eyes with very long axial lengths (≥26 mm) or a high degree of myopic refractive error (≤–6 diopters [D]) are diagnosed as high myopia,6 which is one of the major causes of legal blindness in developed countries.7–9 Highly myopic eyes are often affected by a variety of myopic complications.10 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 20/200 in 89% and in 96% of eyes, respectively.11,12 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,13–19 only limited studies have explored the genetic background of the occurrence of CNV secondary to high myopia. Fernandez-Robredo et al.,20 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.21–23 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.24 In addition, a recent study reported a positive association between the complement factor I (CFI) gene polymorphism, rs10053900, and the occurrence of CNV by using 71 cases and 196 controls in Caucasians.24 These results indicate that genetic background plays a role in CNV observed in AMD and is also secondary to high myopia.

Serpine peptidase inhibitor, clade F (SERPING1), 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.\textsuperscript{25–27} Several groups have evaluated PEDF as a candidate gene for neovascular diseases such as diabetic retinopathy and AMD.\textsuperscript{28–34} Regarding CNV, Lin et al.\textsuperscript{35} 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,\textsuperscript{30,32,34} our group showed that another SNP in PEDF, rs12603825, was associated with the response of polypoidal choroidal vasculopathy to photodynamic therapy (PDT).\textsuperscript{33} 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 ≥50 years of age (mean age ± SD, 66.7 ± 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 ≥ 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 ≥ 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 angiod 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 ≥ 26.00 mm in at least one eye) Japanese individuals who were 50 years of age and older (64.3 ± 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) 50 years of age and older at the first visit with CNV to our institutes. The inclusion criteria for the control group were the same as those for the overall control group.

### 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,\textsuperscript{29,33} 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.\textsuperscript{24}

### 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 ± 1.84 mm and −13.40 ± 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 subanalysis 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 ≥ 28.00 mm in at least one eye, (2) axial length ≥ 29.00 mm in at least one eye, and (3) axial length ≥ 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
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.

**TABLE 1. Characteristics of the Study Population**

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

* 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.

**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. and our group showed no association between the occurrence of CNV secondary to high myopia and ARMS2 or CFH. We also found no association from the evaluation of VEGFA, 15q14, and 15q25.22,23 Recently, Leveziel et al. 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, 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. On the other hand, we previously reported that rs12603825 in PEDF is associated with the response of AMD to PDT.33 Because other SNPs within the PEDF gene have never been reported to be associated with CNV,30,32,34 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,33 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, 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. showed disparity in T allele.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>CNV (+)</th>
<th>CNV (–)</th>
<th>1000 Genome JPT</th>
<th>Nominal $P$</th>
<th>Adjusted $P$†</th>
<th>Adjusted OR (95% CI)</th>
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<td></td>
<td></td>
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<td>$n$</td>
<td>MAF</td>
<td>$n$</td>
<td>MAF</td>
<td>MAF</td>
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<td>PEDF</td>
<td>rs12603825</td>
<td>GG</td>
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<td>0.292</td>
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<td></td>
<td></td>
<td>GA</td>
<td>192</td>
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<td></td>
<td></td>
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<td>CC</td>
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<td>0.483</td>
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<td>0.461</td>
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<td></td>
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<td>TT</td>
<td>114</td>
<td>0.118</td>
<td>118</td>
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<td>CFI</td>
<td>rs10033900</td>
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<td>200</td>
<td>0.357</td>
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<td>0.360</td>
<td>0.354</td>
<td>0.86</td>
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<td></td>
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<td>TC</td>
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<td>0.233</td>
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<td></td>
<td></td>
<td>CC</td>
<td>67</td>
<td>0.76</td>
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</table>

CI, confidence interval.
* Cochran–Armitage test.
† 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, Leveziel et al. 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 FDR 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 it 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 \( (P < 0.05) \), 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

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### References


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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Allele</th>
<th>Axial Length</th>
<th>CNV (+)</th>
<th>CNV (−)</th>
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<td>1/2</td>
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* Logistic regression analysis. Adjusted for age, sex, and axial length.