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Insulin-like growth factor 1 is not associated with high myopia in a large Japanese cohort

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Purpose: To investigate whether genetic variations in the insulin-like growth factor 1 (*IGF-1*) gene are associated with high myopia in Japanese.

Methods: A total of 1,339 unrelated Japanese patients with high myopia (axial length ≥26 mm in both eyes) and two independent control groups were evaluated (334 cataract patients without high myopia and 1,194 healthy Japanese individuals). The mean axial length (mm±SD) in the case group was 29.18±1.85 mm, and the mean spherical equivalent (D±SD) of the phakic eyes was −12.69±4.54 D. We genotyped five tagging single nucleotide polymorphisms (SNPs) in *IGF-1*: rs6214, rs978458, rs5742632, rs12423791, and rs2162679. Chi-square tests for trend, multivariable logistic regression, and haplotype regression analysis were conducted.

Results: We found no significant association between the *IGF-1* SNPs and high or extreme myopia (axial length ≥28 mm in both eyes, 837 subjects) in the additive model, even when compared with the cataract and general population controls, with or without adjustments for age and sex. The evaluation using dominant and recessive models also did not reveal any significant associations. The haplotype analysis with a variable-sized sliding-window strategy also showed a lack of association of *IGF-1* SNPs with high or extreme myopia.

Conclusions: The results of the present study using a Japanese subset do not support the proposal that the *IGF-1* gene determines susceptibility to high or extreme myopia in Caucasians and Chinese. Further studies are needed to confirm our reports in other populations and to identify the underlying genetic determinants of these ocular pathological conditions.

Myopia is a common visual disorder found worldwide and poses major public health concerns, especially in East Asian populations. Myopic eyes with long axial lengths (≥26 mm) or a high degree of myopic refractive error (≤−6 D) are classified as high myopia [1]. High myopia is associated with various ocular complications [2], and these pathological conditions are one of the leading causes of legal blindness in developed countries [3-5]. Therefore, elucidating the pathological mechanisms underlying high myopia and discovering methods for preventing or delaying its onset are important.

Myopia is a complex disease caused by environmental and genetic factors. To date, although many studies have evaluated various candidate genes and susceptible loci of high myopia [6-11], no single gene has been consistently responsible for the condition. In addition to candidate gene studies, a genome-wide approach has also been performed by several groups. Our group previously determined a susceptibility locus for pathological myopia in 2009, using a genome-wide association study [12]. In addition, recent genome-wide association studies have revealed myopia susceptibility loci on chromosome 15 [13,14], and we confirmed that these susceptibility loci are also present in high myopia [15]. However, susceptibility genes for myopia have not yet been determined.

Insulin-like growth factor 1 (*IGF-1*) is similar to insulin in function and structure and is a member of a protein family involved in mediating growth and development. Recently, a single nucleotide polymorphism (SNP) in *IGF-1* was reported to be associated with several types of myopia, including high myopia, in Caucasians [16]. However, these associations were not confirmed by a Polish family study that used single-marker association analysis, a family-based association test, a pedigree disequilibrium test, and haplotype analysis [17]. Nevertheless, subsequent Chinese studies reported a significant association of *IGF-1* polymorphisms with high or extreme myopia; Mak et al. [18] reported an association with high myopia according to haplotype analysis but not single-marker analysis, and Zhuang et al. [19] reported an association with extreme myopia but not with high myopia according to the single-marker and haplotype analyses. The *IGF-1*

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gene is located at a well replicated myopia susceptibility locus, MYP3 [20-23]. Since several previous animal studies indicated that insulin and *IGF-1* were involved in myopia development [24-26], resolving these conflicting results and clarifying whether *IGF-1* polymorphisms are indeed associated with high myopia are essential. In the present study, we conducted a systematic case-control study to validate the association between polymorphisms of the *IGF-1* gene and high and extreme myopia, using a large cohort of 2,867 unrelated Japanese individuals.

METHODS

Subjects: A total of 1,339 unrelated Japanese patients with high myopia who had agreed to participate in genomic study were recruited from the Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, Kobe City Medical Center General Hospital, and Ozaki Eye Hospital. All the patients underwent a comprehensive ophthalmic examination, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography or partial coherence interferometry (IOLMaster, Carl Zeiss Meditec, Dublin, CA). To be classified as having high myopia, the subjects had to have an axial length ≥26 mm in both eyes. Of the 1,339 patients with high myopia, 837 had extreme myopia, which is defined as an axial length ≥28 mm in both eyes.

As control subjects, two cohorts were included. One cohort was composed of selected controls, comprising 334 cataract patients with axial lengths <25.0 mm in both eyes (control 1). These patients were recruited from the Department of Ophthalmology at Kyoto University Hospital, Ozaki Eye Hospital, Japanese Red Cross Otsu Hospital, and Nagahama City Hospital. The axial length was measured with applanation A-scan ultrasonography or partial coherence interferometry before cataract surgery, and dilated fundus examination was performed after surgery. If the fundus examination results revealed myopic changes such as lacquer cracks/peripapillary atrophy, staphyloma, or choroidal neovascularization, the subject was eliminated from control 1. The other cohort was composed of general population controls, comprising 1,194 healthy Japanese individuals recruited from the Aichi Cancer Center Research Institute, who had agreed to participate in genomic study (control 2).

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

DNA extraction: Total genomic DNAs were prepared from 14 ml of venous blood. DNA was purified using a DNA extraction kit (QuickGene-610L, Fujifilm, Minato, Tokyo, Japan).

Single nucleotide polymorphism selection and genotyping: We selected tag SNPs in *IGF-1* based on HapMap Phase II (Build 36) genotype data [27] using the Haploview software (ver. 4.2). Although previous studies tagged relatively minor SNPs (minor allele frequencies [MAFs] ≥0.05 or 0.10 were applied), all such minor SNPs showed no association with high or extreme myopia. In addition, rs6214 and rs12413791, which were reported to be associated with high and extreme myopia, respectively, showed a MAF of 43% and 31% in HapMap JPT (Japanese in Tokyo, Japan). Thus, we tagged all the major (MAF ≥30%) SNPs that showed a Hardy–Weinberg p≥0.05. Using a tagger pairwise program provided in Hapmap project (R2 cutoff of 0.90), we selected five SNPs: rs6214, rs978458, rs5742632, rs12423791, and rs2162679. These tags provided 100% coverage of the major HapMap SNPs within an 84.65-kilobase region spanning the *IGF-1* gene.

The samples of the high myopia cases and cataract controls were genotyped using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). The individuals recruited from the Aichi Cancer Center Research Institute were genotyped using Illumina HumanHap 610 chips (Illumina Inc., San Diego, CA). Because rs12413791 was not included in this chip, the genotype for rs12423791 was imputed using the MACH software, based on the HapMap Phase II JPT genotype data.

Statistical analyses: Deviations in genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed for each group with the chi-square test. The chi-square test for trend or its exact counterpart was used to compare the genotype distributions of the two groups. To adjust for age and sex, we performed a multivariable logistic regression analysis. In addition, dominant and recessive models were also calculated using the chi-square test. These statistical analyses and power calculation were performed with R software (R Foundation R 2.13.0 for Statistical Computing, Vienna, Austria) and PLINK software (ver. 1.07). We also conducted a haplotype analysis by using a variable-sized sliding-window strategy [28] using the PLINK software. p≤0.05 was considered statistically significant. The Bonferroni correction was used for multiple comparisons.

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 ≥26.00 mm in both eyes. Extreme myopia: axial length ≥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 ± standard deviation (SD) of 29.18±1.85 mm. Among these 2,678 eyes, 1,881 (70.2%) were phakic. Their mean refraction was −12.69±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±SD of 22.96±0.87 mm. The mean refraction of the phakic eyes in control 1 was −0.379±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‡
		Control 1		
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
		Control 2		
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 χ^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
2-SNP window		
rs6214-rs978458	0.425	0.497
rs978458-rs5742632	0.572	0.422
rs5742632-rs12423791	0.712	0.447
rs12423179-rs2162679	0.149	0.493
3-SNP window		
rs6214-rs5724632	0.246	0.53
rs978458-rs12423791	0.564	0.336
rs5742632-rs2162679	0.252	0.327
4-SNP window		
rs6214-rs12423791	0.298	0.306
rs978458-rs2162679	0.293	0.343
5-SNP window		
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 (≥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 ≥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 ≥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.

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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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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%).[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*, *HrtA* 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.[23] 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.[24] 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.[25–27] Several groups have evaluated *PEDF* as a candidate gene for neovascular diseases such as diabetic retinopathy and AMD.[28–34] Regarding CNV, Lin et al.[29] 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,[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).[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 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 ≥ 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) the presence of extreme myopia (axial lengths ≥ 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 ≥ 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 ≥ 29.00 mm and ≥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,[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.[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 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 ≥ 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

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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.[20] and our group showed no association between the occurrence of CNV secondary to high myopia and *ARMS2* or *CFH*.[21] We also found no association from the evaluation of *VEGFA*, 15q14, and 15q25.[22,23] Recently, Leveziel et al.[24] 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,[29] 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.[29] 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,[24] 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.[24] showed disparity in T allele

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

			CNV (+)		CNV (–)		1000 Genome JPT	Statistical Analysis		
Gene	SNP	Genotype	n	MAF	n	MAF	MAF	Nominal P*	Adjusted P†	Adjusted OR (95% CI)
PEDF	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					
PEDF	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					
CFI	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 1	Allele 2	Axial Length, mm	CNV (+) 1/1	CNV (+) 1/2	CNV (+) 2/2	CNV (–) 1/1	CNV (–) 1/2	CNV (–) 2/2	Statistical Analysis* Adjusted P Value	Statistical Analysis* 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, Leveziel et al.[24] 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.

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Investigative Ophthalmology & Visual Science

Prevalence and Genomic Association of Reticular Pseudodrusen in Age-Related Macular Degeneration

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• PURPOSE: To survey the prevalence of reticular pseudodrusen in late age-related macular degeneration (AMD) using multiple imaging methods, and to investigate the association between reticular pseudodrusen and polymorphisms in complement factor H (*CFH*) and age-related maculopathy susceptibility 2 (*ARMS2*) genes.

• DESIGN: Retrospective case series.

• METHODS: This study included 216 consecutive patients with late AMD (typical AMD, polypoidal choroidal vasculopathy [PCV], retinal angiomatous proliferation [RAP], or geographic atrophy). Eyes were assessed for reticular pseudodrusen using the blue channel of color fundus photography, infrared reflectance, fundus autofluorescence, and spectral-domain optical coherence tomography. The major AMD-associated single nucleotide polymorphisms (*CFH* Y402 rs1061170, *CFH* I62 V rs800292, and *ARMS2* A69S rs10490924) were genotyped.

• RESULTS: Forty-nine eyes of 30 patients had a reticular pattern in ≥2 imaging modalities and were diagnosed with reticular pseudodrusen. Of these, 16 had bilateral late AMD, whereas 32 of 186 patients without reticular pseudodrusen had bilateral late AMD ($P < .001$). The prevalence of reticular pseudodrusen was 83% in RAP, 50% in geographic atrophy, 9% in typical AMD, and 2% in PCV. The frequency of the T allele in ARMS2 A69S in patients with and without reticular pseudodrusen was 78.6% and 59.9%, respectively ($P = .007$).

• CONCLUSIONS: The prevalence of reticular pseudodrusen was low in PCV cases. About 50% of patients with reticular pseudodrusen had bilateral late AMD. The connection of ARMS2 risk allele and reticular pseudodrusen was confirmed in a Japanese population. (Am J Ophthalmol 2013;155:260–269. © 2013 by Elsevier Inc. All rights reserved.)

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RETICULAR DRUSEN, DESCRIBED IN THE WISCONSIN Grading System as one type of drusen that form ill-defined networks of broad interlacing ribbons, were first identified using blue-light fundus photography.[1,2] Arnold and associates described a yellowish interlacing network of oval-shaped or roundish lesions, termed reticular pseudodrusen, with a diameter of 125–250 μm that were seen in red-free fundus photography and infrared scanning-laser ophthalmoscopy (SLO).[3] Recently, reticular pseudodrusen have been recognized as an additional distinctive morphologic feature observed in age-related macular degeneration (AMD).[4] Furthermore, several reports have suggested that reticular pseudodrusen are associated with a high risk of progression to late AMD.[5–8] In the longitudinal Beaver Dam Eye Study, reticular pseudodrusen were found to confer a high risk of progression to late-stage AMD, with twice the risk compared with eyes with soft drusen.[8]

The development of new imaging methods, such as confocal SLO and spectral-domain optical coherence tomography (SD OCT), has led to improvements in diagnosing reticular pseudodrusen.[4,7–14] Previous reports showed that near-infrared reflectance (IR), fundus autofluorescence (FAF), and SD OCT were more useful than conventional fundus photography to detect reticular pseudodrusen and suggested that the assessment of reticular pseudodrusen should involve multiple imaging methods.[4,7-10,13,14]

Existing evidence suggests an association of AMD with polymorphisms in the complement factor H (*CFH*) gene and age-related maculopathy susceptibility 2 (*ARMS2*) gene.[15-24] Among the various polymorphisms, the Y402H and I62V variants in the *CFH* gene and the A69S variant in the *ARMS2* gene have been reported to show an association with AMD.[15-24] Recently an association between reticular pseudodrusen and polymorphisms in these genes has been reported.[8,25] Klein and associates showed that the prevalence of reticular pseudodrusen was higher in those homozygous (CC) or heterozygous (TC) for *CFH* Y402H than in those without this variant (TT).[8] On the other hand, Smith and associates demonstrated that *CFH* Y402H risk variant was significantly associated with the absence of reticular macular disease but enhanced risk for reticular macular disease was conferred by the *ARMS2* A69S risk allele.[25] Thus, to date, the association between reticular pseudodrusen and genomic

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background has not reached a consensus. In addition, little is known about the distribution of reticular pseudodrusen in each AMD subtype.

The purpose of this study was to survey the prevalence of reticular pseudodrusen in late AMD using multiple imaging methods and, moreover, to investigate the association of high-risk alleles in the *CFH* (Y402H, rs1061170 and I62V, rs800292) and *ARMS2* (A69S, rs10490924) genes with reticular pseudodrusen. Several terminologies have been used to describe this clinical feature.[2,3,9,14] In this report, we use the term "reticular pseudodrusen" according to the nomenclature by Arnold and associates.[3]

METHODS

WE RETROSPECTIVELY REVIEWED THE MEDICAL RECORDS OF 249 consecutive patients with newly diagnosed late AMD who first visited the Macular Service at Kyoto University Hospital between August 3, 2009 and July 21, 2011. Subjects included in this study were ≥50 years of age and had either typical AMD, polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP), or geographic atrophy. The diagnosis of PCV was based on the indocyanine green angiography (IA) showing a branching vascular network terminating in polypoidal swelling. The diagnosis of RAP was based on the criteria of Yannuzzi and associates[26] via fundus photography, fluorescein angiography (FA), IA, and SD OCT. Neovascular AMD other than PCV or RAP was defined as typical AMD. Geographic atrophy was defined using color fundus photography as a sharply delineated area (at least 175 μm in diameter) of hypopigmentation, depigmentation, or apparent absence of the retinal pigment epithelium (RPE) in which choroidal vessels were clearly visible. Eyes with other macular abnormalities (ie, pathologic myopia, idiopathic choroidal neovascularization, presumed ocular histoplasmosis, angioid streaks, other secondary choroidal neovascularization, central serous chorioretinopathy, epiretinal membrane, or retinal arterial macroaneurysm) were excluded from this study. All diagnoses were made by 3 retinal specialists (S.O., K.Y., and A.T.) who observed the images together and discussed each case; however, a fourth specialist (N.Y.) was consulted in case of a disagreement between the 3 initial reviewers. The fourth specialist made a decision in 13 of the 249 patients (5.2%). Patients were included only if at least 3 specialists agreed on the diagnosis.

All study investigations adhered to the tenets of the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board and the Ethics Committee of Kyoto University Graduate School of Medicine prior to the study. Written informed consent was obtained from all patients who were genotyped. Because this was a retrospective study, written informed consent for research participation was not obtained, but the nature of this study was explained on our website.

• MULTIMODAL IMAGING METHODS: All patients underwent a complete ophthalmologic examination, including measurement of best-corrected visual acuity, determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a noncontact lens, color fundus photography, SD OCT, IR, FAF, FA, and IA.

Color fundus photographs (field, 30-40 degrees) were obtained digitally using a Topcon TRC NW6S nonmydriatic retinal camera (Topcon, Tokyo, Japan) after medical dilation of the pupil (phenylephrine 0.5% and tropicamide 0.5%). To examine the blue channel of the color photography, ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) was used to display the individual color channels (red, green, and blue) of the obtained photographs. In ImageJ, the command path of Image > Color > Split Channels was used. Subsequently, the command path of Image > Adjust > Brightness/Contrast was used if needed. Adjustment was performed automatically using the ImageJ software before grading.

IR, FAF, FA, and IA images were acquired using a confocal SLO (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). The IR images were obtained using a light stimulus of 820 nm. The FAF images were obtained using an excitation light of 488 nm and a barrier filter beginning at 500 nm. The field of view was set to 30 × 30 degrees centered on the macula.

SD OCT was conducted using a Spectralis HRA+OCT (Heidelberg Engineering). First, horizontal and vertical line scans through the fovea center were obtained at a 30-degree angle, followed by serial horizontal scans with an examination field size ranging from 30 × 10 degrees to 30 × 25 degrees, depending on the case. At each location of interest on the retina, 50 SD OCT images were acquired and averaged to reduce speckle noise.

• DEFINITION OF THE RETICULAR PSEUDODRUSEN USING MULTIMODAL IMAGING: First, the quality of each image was evaluated by an experienced ophthalmologist (N.U.A.) and patients with adequate image quality in both eyes were included. Image quality was evaluated twice on all other days, and only images having an eligible quality during both evaluations were used. All these images were evaluated for the detection of reticular pseudodrusen by 2 independent experienced ophthalmologists (N.U.A. and S.O.). The evaluation of each image was performed referring to the corresponding images obtained from other imaging modalities. FA images were also referred to in order to distinguish reticular drusen from other lesions such as basal laminar drusen. In case of any discrepancy, a third experienced ophthalmologist (A.T.) was asked to arbitrate. In the current study, eyes diagnosed as having reticular pseudodrusen were those with reticular patterns in more than 2 of the following: the blue channel image

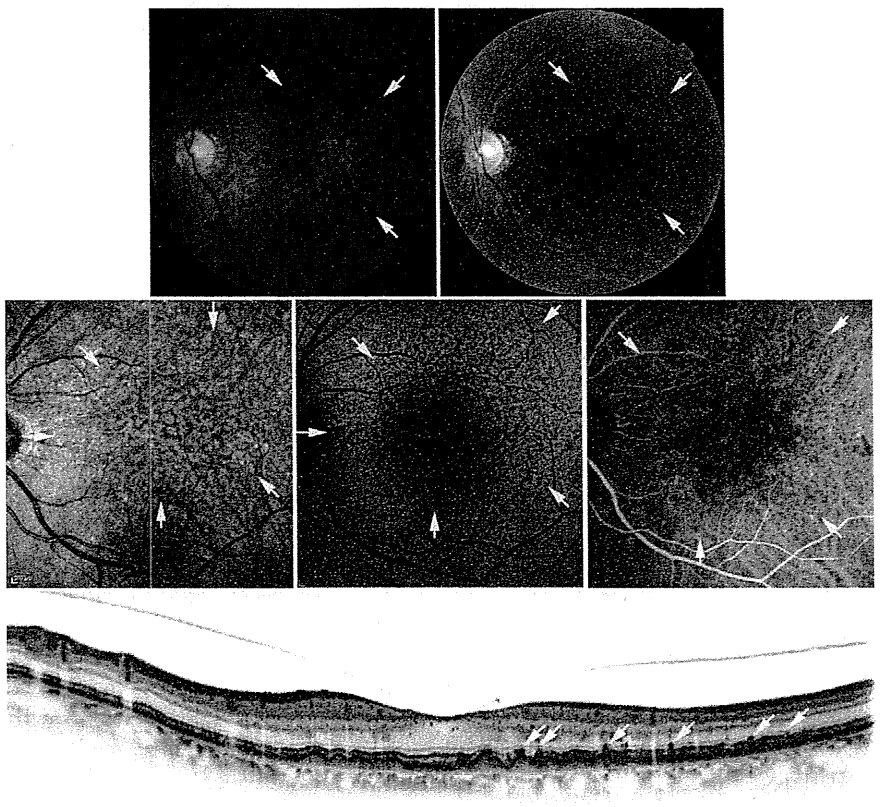

FIGURE 1. Reticular patterns in late age-related macular degeneration in multimodal imaging. (Top row, left) Color fundus photography. (Top row, right) Blue channel of contrast-enhanced color fundus photography. For color fundus or the corresponding blue channel of contrast-enhanced color fundus photography, reticular pattern is identified as light interlacing networks (arrows). (Second row, left) Infrared reflectance (IR). Reticular IR is identified as a grouping of hyporeflectant lesions against a background of mild hyperreflectance with analogous characteristics (arrows). (Second row, middle) Fundus autofluorescence (FAF). Reticular FAF is identified as a grouping of ill-defined, hypofluorescent lesions against a background of mildly elevated FAF (arrows). (Second row, right) Indocyanine green angiography (late phase). A pattern of hyporeflective dots is seen (arrows) corresponding to the reticular pattern in color fundus photography. (Bottom) Spectral-domain optical coherence tomography (SD OCT). Vertical line scan thorough the fovea in the direction of the green arrow in Second row, left shows reticular lesions identified as hyperreflective mounds or triangular lesions above the retinal pigment epithelium (arrows).

of color fundus photography, IR, FAF, or SD OCT. For the blue channel of contrast-enhanced color fundus photography, a reticular pattern was identified as light interlacing networks that were 125-250 μm wide (Figure 1).[3] Reticular autofluorescence was defined as a group of ill-defined, hypofluorescent lesions against a background of mildly elevated AF (Figure 1).[11,13] Reticular IR was defined as a group of hyporeflectant lesions against a background of mild hyperreflectance with analogous characteristics (Figure 1).[14] SD OCT reticular lesions were defined as ≥5 hyperreflective mounds or triangular lesions above the RPE in ≥1 B-scan (Figure 1).[10]

• GENOTYPING: Genomic DNA was prepared from leukocytes of peripheral blood with a DNA extraction kit (QuickGene-610L; Fujifilm, Tokyo, Japan). Of the 216 patients who met the inclusion criteria, genomic data from 11 patients were not available because of the following reasons: (1) consensus of blood extraction was not achieved; (2) genotyping was not possible because of the preservation state. Thus, analyses for genomic data were limited to 205 patients. We genotyped the major AMD-associated single nucleotide polymorphism (SNP), *CFH* Y402 rs1061170, I62 V rs800292, and *ARMS2* A69S rs10490924. The SNPs were genotyped using TaqMan SNP assays with the ABI PRISM 7700 system (Applied Biosystems Inc, Foster City, California, USA), according to the manufacturer's instructions.

• STATISTICAL ANALYSIS: Statistical analysis was performed using SPSS 17 software (SPSS Inc, Chicago, Illinois, USA). All values are presented as a mean ± standard deviation (SD). For statistical analysis, visual acuity measured using a Landolt chart was converted to the logarithm of the minimal angle of resolution (logMAR). Mann-Whitney *U* tests were used to compare data from 2 groups

TABLE 1. Characteristics of Patients With Late Age-Related Macular Degeneration in This Study

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients (%)	97 (44.9)	87 (40.2)	12 (5.6)	12 (5.6)	8 (3.7)	216 (100)
Sex, n (%)						
Men	76 (78)	70 (80)	4 (33)	7 (58)	4 (50)	161 (75)
Women	21 (22)	17 (20)	8 (67)	5 (42)	4 (50)	55 (25)
No. of affected eyes (%)						
Two	17 (18)	9 (10)	5 (42)	9 (75)	8	48 (22)
One	80 (82)	78 (90)	7 (58)	3 (25)	☒	168 (78)
Age (mean ± SD)	74.8 ± 8.3	71.5 ± 8.4	81.3 ± 8.2	72.3 ± 9.3	82.3 ± 2.9	73.9 ± 8.7

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (4 typical AMD and PCV, 1 typical AMD and RAP, 1 typical AMD and geographic atrophy, 1 geographic atrophy and RAP, and 1 geographic atrophy and PCV).

in which normal distributions were not verified. To compare ratios between the 2 groups, χ^2 tests were used. $P < .05$ was considered statistically significant.

RESULTS

IN THIS STUDY, DATA OF 249 CONSECUTIVE PATIENTS WITH late AMD were retrospectively reviewed; however, 5 patients with an eye with phthisis bulbi and 28 patients with poor image quality were excluded. (The intraobserver agreement for grading of image quality was 94.6%.) Thus, 216 patients were included in this study. All patients were Japanese. The patients comprised 161 men and 55 women, aged 51-92 years (mean ± SD, 73.9 ± 8.7). Among them, 97 patients (44.9%) had typical AMD, 87 (40.2%) had PCV, 12 (5.6%) had RAP, and 12 (5.6%) had geographic atrophy. Eight patients had a different type of late AMD in both eyes that was defined as "combined." (Four patients had typical AMD in 1 eye and PCV in the other eye. The other combinations were typical AMD and geographic atrophy, typical AMD and RAP, geographic atrophy and PCV, and geographic atrophy and RAP. The visual acuity of these patients ranged from 20/2000 to 20/12 (mean logMAR = 0.33 ± 0.52). Spherical equivalent refractive error ranged from -5.50 diopters (D) to +4.50 D in the eyes with late AMD, and ranged from -18.375 D to +3.875 D in the fellow eyes without late AMD (4 eyes with high myopia [<-6 D] were included in the fellow eyes). Sixty-eight eyes had pseudophakia. The characteristics of the participants are summarized in Table 1.

Using color fundus photography, IR, FAF, or SD OCT, it was determined that out of 432 eyes, 30 eyes (6.9%), 65 eyes (15.0%), 45 eyes (10.4%), and 47 eyes (10.9%), respectively, had a reticular pattern. (Inter- and intraobserver agreements for grading for the detection of reticular pseudodrusen are shown in Table 2.) Furthermore, 49 eyes (11.3%) of 30 patients had a reticular pattern according to ≥2 imaging modalities, and were defined as having reticular pseudodrusen. Reticular pseudodrusen was confirmed bilaterally in 19 of these 30 patients (63.3%) and unilaterally in 11 patients (36.7%). In all 11 patients with unilateral reticular pseudodrusen, the other eye had neovascular AMD (4 were RAP, 6 were typical AMD, and 1 was PCV). In 38 of 49 eyes (77.6%) with reticular pseudodrusen, a pattern of hyporeflective dots was detected in the middle- and late-phase IA corresponding to the reticular pattern detected in IR and FAF (Figure 1).

The characteristics of patients with reticular pseudodrusen (30 patients [13.9%]) and patients without reticular pseudodrusen (186 patients) are summarized in Tables 3 and 4, respectively. Of the 30 patients with reticular pseudodrusen, 19 (63.3%) were women, whereas only 36 of 186 patients without reticular pseudodrusen (19.4%) were women ($P < .001$, χ^2 test). The mean age of the patients with reticular pseudodrusen was 80.6 ± 6.8 years (range, 65-92 years), which was significantly higher than that of patients without reticular pseudodrusen (72.8 ± 8.5 years; range, 51-92 years; $P < .001$, Mann-Whitney test). In addition, 16 of 30 patients with reticular pseudodrusen (53.3%) had bilateral late AMD, whereas only 32 of 186 patients without reticular pseudodrusen (17.2%) had bilateral late AMD ($P < .001$, χ^2 test). In patients over 70 years old, 15 of 28 patients with reticular pseudodrusen (53.6%) had bilateral late AMD, whereas 26 of 118 patients without reticular pseudodrusen (22.0%) had bilateral late AMD ($P = .001$, χ^2 test).

The prevalence rate of reticular pseudodrusen was different according to the disease type of late AMD: 10 of 12 patients with RAP (83.3%), 6 of 12 patients with geographic atrophy (50.0%), 9 of 97 patients with typical AMD (9.2%), 2 of 87 patients with PCV (2.2%), and 3 of 8 patients with combined subtype (Table 5). In patients over 70 years old, the prevalence of reticular pseudodrusen was 10 of 11 patients with RAP (90.9%), 5 of 7 patients

TABLE 2. Intra- and Interobserver Agreements for Grading of the Detection of Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Blue Channel[a]	IR	FAF	SD OCT
Intraobserver agreements				
Accordance rate (%)	96.9	94.6	95.3	98.3
Kappa coefficient (95% confidence interval)	0.72 (0.57-0.86)	0.78 (0.69-0.86)	0.74 (0.63-0.85)	0.91 (0.85-0.98)
Interobserver agreements				
Accordance rate (%)	94.7	91.4	91.2	97.9
Kappa coefficient (95% confidence interval)	0.62 (0.47-0.76)	0.64 (0.54-0.75)	0.61 (0.50-0.72)	0.89 (0.81-0.96)

FAF = fundus autofluorescence; IR = near-infrared reflectance; SD OCT = spectral-domain optical coherence tomography.
[a]Blue channel of color fundus photography.

TABLE 3. Characteristics of Patients With Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients	9	2	10	6	3	30
Sex, n (%)						
Men	5 (56)	0	3 (30)	2 (33)	1 (33)	11 (37)
Women	4 (44)	2 (100)	7 (70)	4 (67)	2 (67)	19 (63)
No. of eyes with AMD (%)						
Two	3 (33)	0 (0)	4 (40)	6 (100)	3	16 (53)
One	6 (67)	2 (100)	6 (60)	0 (0)		14 (47)
Age (mean ± SD)	79.1 ± 6.6	82.0 ± 4.2	82.4 ± 7.7	78.3 ± 8.0	83.0 ± 2.6	80.6 ± 6.8

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.
[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (1 RAP and GA, 1 AMD and RAP, and 1 AMD and PCV).

with geographic atrophy (71.4%), 8 of 67 patients with typical AMD (11.9%), and 2 of 53 patients with PCV (3.8%) (Supplemental Table, available at AJO.com).

In 28 patients with reticular pseudodrusen and 177 patients without reticular pseudodrusen, the frequency of the minor allele in *CFH* I62 V polymorphism was 21.4% and 23.0%, respectively (Table 6). Upon analyzing the genotype, we determined the G allele did not contribute to reticular pseudodrusen ($P = .865$). The frequency of the C allele in *CFH* Y402H was 14.3% and 16.7%, respectively, in the patients with and without reticular pseudodrusen (Table 6). The 2×2 table from the allele χ^2 test revealed no C allele contribution to reticular pseudodrusen ($P = .845$).

In contrast, the A69S polymorphism in the *ARMS2* gene apparently contributed to reticular pseudodrusen (Table 6). The frequency of homozygosity for the at-risk genotype (TT) of A69S was 60.7% and 38.4%, respectively, in patients with and without reticular pseudodrusen. Furthermore, the frequency of the T allele in A69S was 78.6% and 59.9%, respectively. When examined with a 2×2 table from the allele χ^2 test, we found the T allele contributed significantly to reticular pseudodrusen ($P = .007$).

DISCUSSION

RETICULAR PSEUDODRUSEN HAS TRADITIONALLY BEEN identified with blue-light fundus photography. However, with the development of various imaging modalities, recent studies have suggested that additional methods such as FAF, IR imaging, and SD OCT would facilitate the identification of reticular pseudodrusen.[4,5,7,9,10,13,14] In all these cited studies, multiple imaging modalities were used to detect reticular pseudodrusen, with diagnosis based on the least positive modality. However, the reticular pattern is sometimes subtle and difficult to distinguish from other alterations (soft/hard drusen) when using only 1 imaging modality (Figure 2). Therefore, we used 4 imaging methods. Reticular pseudodrusen-affected eyes were defined as those with reticular patterns discovered with the use of >2 imaging modalities. Among these 4 modalities, IR showed the highest sensitivity for detecting reticular pseudodrusen, consistent with previous studies.[9,14]

Previous reports suggest that IA is also useful for detecting reticular pseudodrusen.[14,27,28] Here, we reviewed IA images from patients with reticular pseudodrusen. In many eyes, hyporeflective dots were detected in the

TABLE 4. Characteristics of Patients Without Reticular Pseudodrusen in Late Age-Related Macular Degeneration

	Typical AMD	PCV	RAP	Geographic Atrophy	Combined[a]	Total
No. of patients	88	85	2	6	5	186
Sex, n (%)						
Men	71 (81)	70 (82)	1 (50)	5 (83)	3 (60)	150 (81)
Women	17 (19)	15 (18)	1 (50)	1 (17)	2 (40)	36 (19)
No. of eyes with AMD (%)						
Two	14 (16)	9 (11)	1 (50)	3 (50)	5 (100)	32 (17)
One	74 (84)	76 (89)	1 (50)	3 (50)	☒	154 (83)
Age (mean ± SD)	74.2 ± 8.3	71.3 ± 8.4	76.0 ± 11.3	66.2 ± 6.2	81.8 ± 3.3	72.8 ± 8.5

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation; SD = standard deviation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (1 RAP and geographic atrophy, 1 AMD and RAP, and 1 AMD and PCV).

TABLE 5. Prevalence of Reticular Pseudodrusen in Each Disease Type of Late Age-Related Macular Degeneration

	No. of Patients	No. of Patients With Reticular Pseudodrusen	Prevalence (%)
Typical AMD	97	9	9.2
PCV	87	2	2.2
RAP	12	10	83.3
Geographic atrophy	12	6	50.0
Combined[a]	8	3	37.5

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; RAP = retinal angiomatous proliferation.

[a]Patients with typical AMD, PCV, RAP, or geographic atrophy in 1 eye and another type of AMD in the other eye (4 typical AMD and PCV, 1 typical AMD and RAP, 1 typical AMD and geographic atrophy, 1 geographic atrophy and RAP, and 1 geographic atrophy and PCV).

middle and late phases of reticular pseudodrusen development. These dots correspond to the reticular pattern detected in IR and FAF. Smith and associates reported that IA detected reticular pseudodrusen with 100% sensitivity, although the sample size was small.[14] Our findings suggest that IA is a useful method to detect reticular pseudodrusen.

In our study, the proportion of women was higher in the reticular pseudodrusen group than in the group without reticular pseudodrusen. Patients with reticular pseudodrusen were older than those without. These results are consistent with previous reports indicating that women and older patients are more likely to have reticular pseudodrusen.[3,4,10,14] In addition, many reticular pseudodrusen patients in our study had bilateral late AMD. Arnold and associates reported that CNV was found in 66 of 100 patients with reticular pseudodrusen; bilateral CNV was found in 24 patients.[3] Pumariega and associates showed that reticular pseudodrusen was associated with progression to late AMD in the fellow eye.[6] Thus, ophthalmologists should be aware that patients with reticular pseudodrusen have the risk of bilateral late AMD and should conduct follow-ups on these patients.

The prevalence of reticular pseudodrusen has been described in several reports. A population-based study revealed that the overall prevalence was 0.7% in the general population. The associated 15-year incidence increased from 0.4% to 6.6% with age, which is similar to the trend observed for AMD.[8] In AMD patients, the prevalence of reticular pseudodrusen ranged from 9% to 36%.[3,4,10,13] In the current study, reticular pseudodrusen was detected in 14% of patients with late AMD. This small number may be explained by the following reasons: the diagnosis of reticular pseudodrusen was based on reticular patterns as observed in ≥2 imaging modalities; our cohort included many patients with PCV, in whom reticular pseudodrusen was rarely detected; our subjects were younger (mean age, 73.9 years) than the subjects studied by Cohen and associates (mean age, 79.5 years) [4]; and our study had relatively fewer women than those in previous reports, although the sex distribution was similar to that of other Japanese AMD studies.[29] Considering that women are more likely than men to have reticular pseudodrusen, sex distribution may be one of the reasons for low prevalence of reticular pseudodrusen. Reticular pseudodrusen may fade with post-CNV development.[14] Smith and associates included only the fellow eyes of patients with unilateral CNV.[13] The current study included patients with bilateral CNV; some of these patients may have had reticular pseudodrusen before CNV development. We speculate that there may be ethnic differences in the reticular pseudodrusen prevalence between Japanese and white populations, similar to the varying prevalence of soft drusen among AMD patients.[30-32] A recent Korean study indicated that ethnic differences may be associated with certain clinical features.[33]