Retina

The Contribution of Genetic Architecture to the 10-Year Incidence of Age-Related Macular Degeneration in the Fellow Eye

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PURPOSE. To correlate a genetic risk score based on age-related macular degeneration (AMD) susceptibility genes with the risk of AMD in the second eye.

METHODS. This is a retrospective, open cohort study consisting of 891 unilateral AMD patients, who were followed for at least 12 months and recruited from three institutes. DNAs were genotyped using Illumina OmniExpress, HumanOmni2.5-8, and/or HumanExome. Survival analyses and Cox proportional hazard models were used to examine the association between 11 AMD susceptibility genes and the duration until second-eye involvement in 499 samples from Kyoto University, which were replicated in two other cohorts. Genetic risk score (GRS) was also evaluated.

RESULTS. The *ARMS2* rs10490924 recessive model (hazard ratio [HR]$_{meta}$ = 2.04; P_{meta} = 3.4 × 10^{-3}) and *CFH* rs800292 additive model (HR_{meta} = 1.77; P_{meta} = 0.013) revealed significant associations with second-eye involvement. The dominant model of *TNFRSF10A* rs13278062, *VEGFA* rs943080, and *CFI* rs4698775 showed consistent effects across three datasets (I^2 = 0%; HR_{meta} = 1.46, 1.30, 1.51, respectively). The GRS using these five single nucleotide polymorphisms (SNPs) was also significantly associated (HR_{meta} [per score] = 2.42; P = 2.2 × 10^{-5}; I^2 = 0%). After 10 years from the first visit, the patients within the top 10% by GRS showed a 51% hazard rate, in contrast to 2.3% among patients within the lowest 10% by GRS.

CONCLUSIONS. We demonstrated that the GRS using *ARMS2*, *CFH*, *TNFRSF10A*, *VEGFA*, and *CFI* was significantly associated with second-eye involvement. Genetic risk has high predictive ability for second-eye involvement of AMD.

Keywords: age-related macular degeneration, genetics, second-eye involvement, bilateral AMD

Age-related macular degeneration (AMD) is a major cause of progressive, irreversible visual impairment among elderly populations in developed countries.[1–3] Although the impairment of quality of life (QOL) is limited when AMD has been observed in a single eye, QOL will be severely damaged if the second eye is also affected by AMD.[4,5] Thus, predicting second-eye involvement of AMD in unilateral cases is of great importance in the clinical setting. So far, while many population-based studies have investigated risk factors for AMD in the first eye, reporting age, cataract, education, sex, smoking, dietary intake, and soft drusen as possible risk factors as well as genetic risk,[6–12] risk factors for AMD in the second eye have not been fully evaluated.[13–16]

In 2013, the largest genome-wide association study on AMD identified 19 AMD susceptibility loci,[17] including 12 known loci.[18–32] Some of these genes, together with other environmental factors, were proven to be good predictors for AMD development in the first eye by evaluating a prospective cohort with a mean follow-up of 6.3 years.[13,14] On the other hand, to what extent these AMD susceptibility genes contribute to second-eye involvement is currently controversial. While cross-sectional evaluation[14,33–36] and retrospective longitudinal analysis[15] revealed genetic architecture associated with second-eye involvement, a Comparison of Age-related Macular Degeneration Treatment Trial (CATT) study group reported in their subanalysis of a prospective study that they could not find significant associations between the four AMD susceptibility

genes *CFH*, *ARMS2/HTRA1*, and *C3* and hazard rate of second-eye involvement.[16] However, though the evidence in the prospective study is high, the CATT study is limited by a follow-up period of only 2 years, which might not be long enough to detect the differences across genotypes. In addition, only four single nucleotide polymorphisms (SNPs) were evaluated in the study. Considering the trend observed in the CATT study that risk allele carriers were more likely to develop AMD in their second eye, the impact of genetics on the second-eye involvement needs to be further evaluated.

In the current study, we conducted survival analysis according to the genotypes of 11 out of 19 recently reported AMD susceptibility genes whose associations with AMD have also been confirmed in the Asian population.

MATERIALS AND METHODS

The current study is a multicenter, retrospective, open cohort study, which was approved by the Institutional Review Board at Kyoto University Graduate School of Medicine, Fukushima Medical University, and Kobe City General Hospital, and all study conduct adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient who was genotyped.

Subjects and Phenotyping

All subjects were recruited from a Kyoto AMD cohort that consisted of 1576 unrelated neovascular AMD patients who were genome scanned. These samples were collected from all over Japan, mainly Kyoto University Hospital (Kyoto samples), Fukushima Medical University (Fukushima samples), and Kobe City General Hospital (Kobe samples). Comprehensive ophthalmic examinations were conducted on all patients; these included dilated fundus examination, fluorescein angiography, and indocyanine angiography. The diagnosis of exudative AMD was made by retinal specialists according to the International Classification System for age-related maculopathy[37] as we previously described.[31] Patients with the following conditions were excluded from the study subjects: high myopia (spherical equivalent $\leq$ −6.00 diopters [D]), geographic atrophy or drusen only, and an old lesion without a clear diagnosis of AMD.

For the discovery stage, we selected participants as follows. (1) Quality control (QC) using genotype call rate (details are described in genotyping section) excluded 21 patients, resulting in 1555 patients left. (2) Eight hundred ninety patients who visited Kyoto University Hospital were selected in order to access their detailed charts. (3) A total of 499 patients who had unilateral AMD at their first visit and could be followed for at least 12 months were finally included in this study (Kyoto samples). For the replication study, we included 263 patients who visited Fukushima Medical University (Fukushima samples) and 129 patients who visited Kobe City General Hospital (Kobe samples).

We obtained phenotypic information of these patients by reviewing their charts. The date of occurrence of AMD in the second eye was regarded as the date when the physicians documented AMD newly developing in the fellow eye. The duration from their first visit to event (developing AMD in their second eye) or censoring (final visit in March 2014, or lost to follow-up) was reported on a monthly basis. Age at their first visit was employed as their age for statistical analysis.

Genotyping

Genotyping was performed using Illumina BeadChip (San Diego, CA, USA); 974 individuals were genotyped using both

OmniExpress and HumanExome, 574 individuals using Human-Omni2.5-8, and 28 individuals using OmniExpress. After a stringent QC, including Hardy-Weinberg equilibrium, $P \geq 1.0 \times 10^{-6}$, minor allele frequency (MAF) $\geq$ 1%, and genotype call rate $\geq$ 95% (per SNP and per individual), we imputed them with reference to 1000 Genomes project cosmopolitan haplotypes (1092 samples from all over the world; available at the IMPUTE2 website [https://mathgen.stats.ox.ac.uk/impute/impute_v2.html]; released December 2013; in the public domain) in order to produce constant genotype data across each platform. Quality control was performed using PLINK ver. 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/; in the public domain), while imputation was performed using SHAPEIT2 (http://www.shapeit.fr/; in the public domain) and IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html; in the public domain). Imputation status and quality are described in Supplementary Table S1.

Gene and SNP Selection

From the 19 genes that were significantly associated with AMD in the report by the AMD Gene Consortium, we used 11 genes for which associations with AMD were verified in Asian individuals.[17,38] For *CFH*, *C3*, and *CETP*, we employed SNPs that had been commonly evaluated in Asians[30,39–42] instead of the actual SNPs reported by the AMD Gene Consortium. Finally, the following SNPs were evaluated in this study: *ARMS2* (rs10490924), *CFH* (rs800292), *C2/CFB* (rs429608), *C3* (rs2241394), *APOE* (rs4420638), *CETP* (rs3764261), *VEGFA* (rs943080), *TNFRSF10A* (rs13278062), *CFI* (rs4698775), *TGFBR1* (rs334353), and *ADAMTS9* (rs6795735).

Statistical Analysis

Survival analysis on the AMD-free period of the non-AMD eyes in unilateral AMD cases was conducted using the Kaplan-Meier method. A nonrisk allele for AMD was set as the reference allele in all statistical analyses. Cox proportional hazard regression analysis was conducted to adjust for age at first visit and sex. In the first stage, each additive, dominant, and recessive model was tested for each of the 11 genes using Kyoto samples ($n = 499$). The associations that showed a P value less than 0.50 were carried over to the second stage, and we conducted replication studies using the other two cohorts, Fukushima samples ($n = 263$) and Kobe samples ($n = 129$). Results of the Cox regression analyses were meta-analyzed using the inverse-variance method. To evaluate the heterogeneity of the associations across three datasets, we also calculated I^2 value. The hereditary models that showed consistent effects across all three datasets (I^2 value of 0% in meta-analyses) were selected for the final models.

A P value less than 0.05 was considered statistically significant. These statistical analyses were conducted using R software ver. 3.02 (http://www.r-project.org/; in the public domain). CRAN package "survival" was applied.

Genetic Risk Score

We computed genetic risk scores (GRS) using the five genotypes of five genes, which were consistently associated with an AMD-free period across three datasets, namely, *ARMS2* rs10490924 (A69S), *CFH* rs800292 (I62V), *TNFRSF10A* rs13278062, *VEGFA* rs943080, and *CFI* rs4698775 (see Results section for detail). First, we constructed a Cox proportional hazard regression model including these five SNPs at once within Kyoto samples. We employed a recessive model for *ARMS2*, an additive model for *CFH*, and a dominant model for others. As such, we coded the genotypes as follows: risk

TABLE 1. Baseline Characteristics of the Study Subjects

Item	Kyoto	Fukushima	Kobe
n	499	263	129
Sex, male:female	348:151	197:66	89:40
Age at the first visit, y*	72.2 ± 8.2	72.1 ± 8.2	71.6 ± 8.6
Follow-up period, mo†	55.5 (36.0, 83.0)	63.0 (43.0, 84.0)	67.0 (52.0, 95.0)
Number of events	54, 10.8%	22, 8.4%,	6, 4.6%

* Mean ± standard deviation are described.
† Median (1st quantile, 3rd quantile) are displayed.

homos as 1 and others 0 for *ARMS2*, 0 to 2 according to the number of risk alleles for *CFH*, and nonrisk homos as 0 and others 1 for *TNFRSF10A*, *VEGFA*, and *CFI*. Next, based on the single-SNP β coefficients estimated using the Cox regression model, the GRS was calculated as

$$\text{GRS} = \sum_{i=1}^{k} b_{i*}x_i,$$

with k being the number of SNPs in the model, x_i the genotype code of the ith SNP, and b_i the β coefficients for the ith SNP, with reference to the previous reports.[14,43] Finally, the association between GRS and the duration until the development of AMD in the second eye was tested using Kyoto samples and replicated using the independent sample sets of Fukushima and Kobe.

RESULTS

The baseline statistics of the three longitudinal cohorts are summarized in Table 1. The distribution of age at the first visit and male versus female sex was similar among the three cohorts. The median follow-up period ranged from 55.5 to 67.0 months.

Kaplan-Meier curves according to the genotype of the 11 SNPs and corresponding P values before adjustment are shown in Figure 1 and Supplementary Figure S1. This analysis showed that *ARMS2* rs10490924 ($P = 0.028$) and *CFH* rs800292 ($P = 0.029$) were significantly associated with the AMD-free period of the second eye, while marginal associations were observed in *C2/CFB* rs429608 ($P = 0.088$). Though analysis of *ADAMTS9* resulted in a curve similar to that for these three SNPs, we could not find a significant or marginal association ($P = 0.13$). These results showed that 39.8% of the patients who were

homozygous for *ARMS2* rs10490924 developed AMD in their second eye within 10 years, and 27.4% of the patients who were homozygous for *CFH* rs800292 did so. Since only one patient was homozygous for *C2/CFB* rs429608 or *APOE* rs4420635, we evaluated these two SNPs in a recessive model (i.e., risk homozygous versus others). Similarly, *C3* rs2241394 was evaluated in a dominant model (i.e., nonrisk homozygous versus others). The P values of the log-rank tests are displayed in each plot.

Table 2 shows the results of the Cox proportional hazard model for all the hereditary models for the 11 SNPs after adjustment for age at first visit and sex. Strong to modest effects were observed in all hereditary models of *ARMS2*, *CFH*, and *C2/CFB*, six of which yielded a P value of less than 0.05. An additional six associations also showed modest (i.e., odds ratio [OR] ≥ 1.3) effects, with a P value of less than 0.5: *CETP* recessive model, *VEGFA* dominant model, *TNFRSF10A* dominant model, *CFI* dominant model, and *ADAMTS9* additive and recessive models. These associations were further evaluated using two independent Japanese cohorts. Although none of the associations yielded a P value of less than 0.05 in the replication cohorts, possibly due to sample size, six associations of five genes showed consistent effects across all the three datasets with an I^2 value of 0% in meta-analysis, namely, the *ARMS2* recessive model, *CFH* additive and recessive model, *VEGFA* dominant model, *TNFRSF10A* dominant model, and *CFI* dominant model (Table 3). The P value for proportional hazard assumption testing was <0.05 only in the dominant model of rs13278062 in the evaluation of Kobe samples (Supplementary Table S2).

The Cox proportional hazard model, including five genes at once, provided single-SNP β coefficient for each gene (Table 4), and we computed the GRS based on these values as indicated in the Methods section (Table 5). Figure 2 illustrates a Kaplan-

TABLE 2. Hazard Ratio After an Adjustment for Sex and Age at the First Visit Using the Cox Proportional Hazard Model

Gene	SNP	Chr	Position	Effect Allele*	EAF	Additive Model HR per Allele (95% CI)	P	Dominant Model HR (95% CI)	P	Recessive Model HR (95% CI)	P
ARMS2	rs10490924	10	124214448	T	0.58	1.63 (1.06–2.50)	0.025	1.70 (0.74–3.87)	0.21	2.14 (1.17–3.95)	0.014
CFH	rs800292	1	196642233	G	0.74	2.10 (1.15–3.81)	0.016	3.71 (0.51–27.1)	0.20	2.30 (1.15–4.58)	0.018
C2/CFB	rs429608	6	31930462	G	0.94	0.43 (0.21–0.90)	0.025	—	—	0.42 (0.20–0.88)	0.022
C3	rs2241394	19	6685230	C	0.07	0.95 (0.40–2.24)	0.91	0.96 (0.40–2.28)	0.93	—	—
APOE	rs4420638	19	45422946	T	0.93	1.13 (0.44–2.87)	0.80	—	—	1.12 (0.44–2.87)	0.81
CETP	rs3764261	16	56993324	A	0.22	0.87 (0.50–1.50)	0.61	0.73 (0.39–1.38)	0.33	1.74 (0.53–5.65)	0.36
VEGFA	rs943080	6	43826627	T	0.33	1.04 (0.67–1.62)	0.86	1.44 (0.51–4.08)	0.49	0.93 (0.50–1.72)	0.82
TNFRSF10A	rs13278062	8	23082971	T	0.40	1.14 (0.74–1.75)	0.55	1.39 (0.72–2.68)	0.32	0.93 (0.39–2.20)	0.86
CFI	rs4698775	4	110590479	G	0.24	1.14 (0.71–1.84)	0.58	1.48 (0.80–2.72)	0.21	0.35 (0.05–2.53)	0.30
TGFBR1	rs334353	9	101908365	T	0.50	1.02 (0.67–1.54)	0.94	0.85 (0.43–1.67)	0.64	1.21 (0.63–2.34)	0.56
ADAMTS9	rs6795735	3	64705365	T	0.87	1.83 (0.84–4.00)	0.13	—	—	1.80 (0.80–4.07)	0.16

Chr, chromosome; EAF, effect allele frequency.
* Risk allele for age-related macular degeneration set to the effect allele.
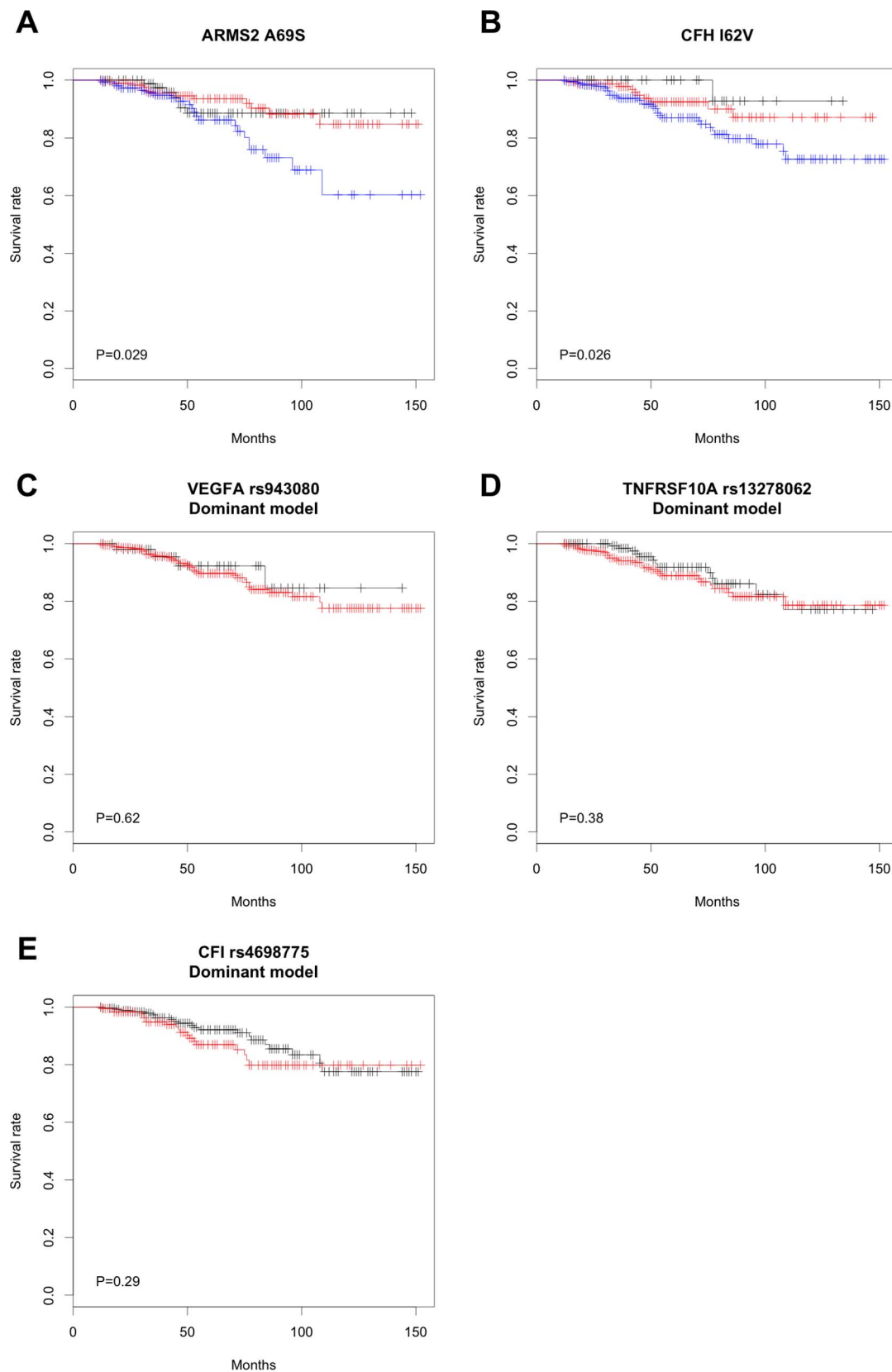
FIGURE 1. Kaplan-Meier curves based on the genotype of each gene in the discovery stage and the corresponding *P* value of the Cox proportional hazard model without adjustment are displayed. (A) Significant differences were observed for *ARMS2* A69S (rs10490924) genotypes. Homozygous patients (TT genotype; *blue*) had a 39.8% hazard rate after 10 years from their first visit. *Red* represents the GT genotype, while *black* represents the GG genotype. (B) Marginal differences were observed among *CFH* I62V (rs800292) genotypes. Homozygous patients (GG genotype; *blue*) had a 27.4% hazard rate after 10 years from their first visit. *Red* represents the GA genotype, while *black* represents the AA genotype. (C) The difference between *VEGFA* rs943080 TT or TC genotypes (*red*) and the CC genotype (*black*) was not statistically significant. (D) The difference between *TNFRSF10A* rs13278062 GT or TT genotype (*red*) and GG genotype (*black*) was not statistically significant. (E) The difference between *CFI* rs4698775 GG or GT genotype (*red*) and the TT genotype (*black*) was not statistically significant.

TABLE 3. Results of the Replication Studies and Meta-Analysis

Gene	SNP	Hereditary Model	First Stage, Kyoto		Replication 1, Fukushima		Replication 2, Kobe		Meta-Analysis		
			HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	I^2
ARMS2	rs10490924	Additive	1.63 (1.06–2.50)	0.025	1.18 (0.62–2.26)	0.61	0.71 (0.23–2.20)	0.56	1.38 (0.99–1.95)	0.060	5.6%
		Dominant	1.70 (0.74–3.87)	0.21	0.51 (0.20–1.34)	0.17	0.44 (0.08–2.50)	0.36	0.93 (0.52–1.67)	0.80	52.7%
		Recessive	2.14 (1.17–3.95)	0.014	2.27 (0.95–5.38)	0.064	0.91 (0.16–5.22)	0.92	2.04 (1.27–3.30)	3.4×10^{-3}	0%
CFH	rs800292	Additive	2.10 (1.15–3.81)	0.016	1.42 (0.66–3.05)	0.36	1.46 (0.31–6.96)	0.64	1.77 (1.13–2.78)	0.013	0%
		Dominant	3.71 (0.51–27.1)	0.20	1.57 (0.21–11.9)	0.67	NA*		2.43 (0.59–10.1)	0.22	(0%)†
		Recessive	2.30 (1.15–4.58)	0.018	1.53 (0.62–3.82)	0.36	1.36 (0.25–7.54)	0.72	1.91 (1.13–3.23)	0.015	0%
C2/CFB	rs429608	Additive	0.43 (0.21–0.90)	0.025	1.47 (0.35–6.23)	0.60	NA*		0.56 (0.29–1.07)	0.078	(54.4%)†
		Recessive	0.42 (0.20–0.88)	0.022	1.46 (0.34–6.31)	0.62	NA*		0.54 (0.28–1.05)	0.071	(54.4%)†
CETP	rs3764261	Recessive	1.74 (0.53–5.65)	0.36	0.92 (0.21–4.04)	0.92	No event in high-risk group		1.36 (0.54–3.41)	0.52	(0%)†
VEGFA	rs943080	Dominant	1.44 (0.51–4.08)	0.49	1.51 (0.20–11.6)	0.69	No event in low-risk group		1.46 (0.58–3.68)	0.43	0%
TNFRSF10A	rs13278062	Dominant	1.39 (0.72–2.68)	0.32	1.06 (0.43–2.65)	0.90	1.67 (0.30–9.4)	0.56	1.30 (0.78–2.16)	0.31	0%
CFI	rs4698775	Dominant	1.48 (0.80–2.72)	0.21	1.61 (0.68–3.8)	0.28	1.40 (0.28–6.98)	0.69	1.51 (0.94–2.43)	0.089	0%
ADAMTS9	rs6795735	Additive	1.83 (0.84–4.00)	0.13	0.84 (0.38–1.85)	0.66	1.40 (0.20–9.62)	0.73	1.25 (0.71–2.17)	0.40	46.9%
		Recessive	1.80 (0.80–4.07)	0.16	0.70 (0.28–1.74)	0.44	1.26 (0.14–11.2)	0.84	1.10 (0.65–2.17)	0.56	56.6%

EAF, effect allele frequency.

* Only one sample was classified to either arm.

† I^2 values of the SNPs for which only two cohorts were meta-analyzed are presented with parentheses. I^2 for VEGFA rs943080 is shown without parenthesis because the third cohort showed the same direction though only two cohorts were meta-analyzed.

TABLE 4. Cox Proportional Hazard Modeling Using Possible Susceptibility Genes in Kyoto Samples

	Hereditary Model	β	SE	P
rs10490924	Recessive	0.787	0.314	0.012
rs800292	Additive	0.757	0.315	0.016
rs943080	Dominant	0.337	0.530	0.53
rs13278062	Dominant	0.216	0.338	0.52
rs4698775	Dominant	0.400	0.313	0.20
Sex, female	—	−0.767	0.381	0.044
Age at first visit	—	0.096	0.023	2.13×10^{-5}

Meier curve based on the GRS. Patients with GRS values within the top 10% from the Kyoto samples had a 51.0% hazard rate after 10 years from the time of their first visit, in contrast to 2.3% among patients with GRS values in the lowest 10% (Fig. 2A). The same trend was observed in the Fukushima samples, in which patients with GRS values in the top 25% had a 27.6% hazard rate, and patients with GRS values in the lowest 25% had a 1.7% hazard rate (Fig. 2B). In these analyses, we used different cutoff values of GRS to assign a similar number of patients to the higher/lower GRS groups. In the Kyoto samples, the top 10% GRS value corresponded to 2.85, and the lower 10% corresponded to 1.10.

The GRS was significantly associated with the time lapse until second-eye involvement in the Kyoto samples after an adjustment for age and sex ($P = 1.81 \times 10^{-4}$), and this was replicated in the Fukushima samples ($P = 0.031$). Though not statistically significant in the Kobe samples, GRS still exhibited a consistent effect, allowing meta-analysis of all three datasets to achieve lower P value ($P_{meta} = 2.22 \times 10^{-5}$) with a hazard ratio (HR) of 2.42 per score, after adjusting for sex and age at the first visit. Patients with low GRS were less likely to develop AMD in their second eye for their age (Fig. 3), while patients with high GRS tended to develop AMD in their second eye earlier for their age (Fig. 4).

DISCUSSION

Since bilateral AMD severely impairs QOL,[4,5] second-eye AMD development is a great concern for clinicians treating patients with unilateral AMD. Bilateral advanced disease was evaluated previously for 5 number of genes. There are few studies that suggest genetic effects on second-eye involvement. In the current study, we demonstrated that GRS calculated using five AMD susceptibility genes, that is, ARMS2, CFH, TNFRSF10A, VEGFA, and CFI, were significantly associated with second-eye involvement, and patients with low GRS values had low hazard rates in contrast to the high hazard rates observed among patients with high GRS.

Though there are studies regarding AMD bilaterality,[44–46] studies evaluating the association between genetics and incidence of bilateral involvement among unilateral late AMD

TABLE 5. Effect of Genetic Risk Score on Second-Eye Involvement of AMD Determined Using the Cox Proportional Hazard Model

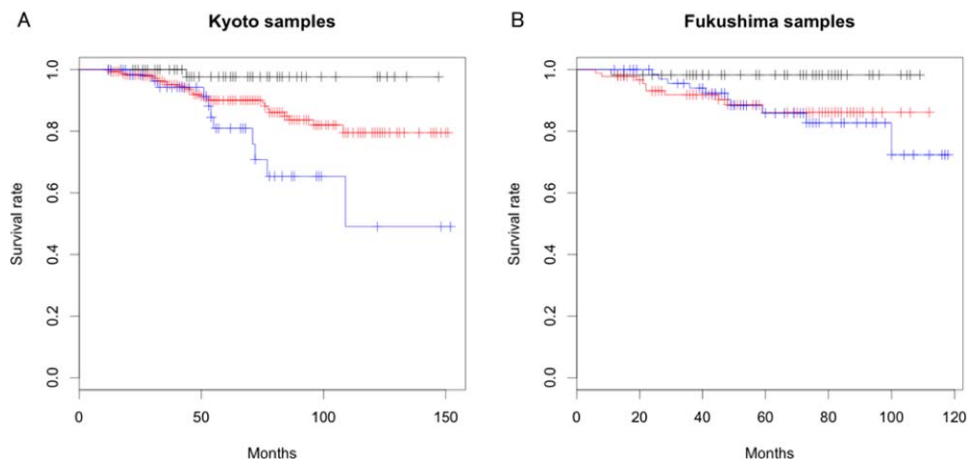|	Genetic Risk Score, per 1 Point		
	HR (95% CI)	P Value	I^2
Kyoto samples	2.72 (1.61–4.59)	1.81×10^{-4}	—
Fukushima samples	2.32 (1.08–4.99)	0.031	—
Kobe samples	1.40 (0.41–4.79)	0.59	—
Meta analysis	2.42 (1.61–3.63)	2.22×10^{-5}	0%

FIGURE 2. Kaplan-Meier curves based on the genetic risk score (GRS) values calculated using the genotypes of *ARMS2*, *CFH*, *TNFRSF10A*, *VEGFA*, and *CFI* are displayed. (**A**) The diagram shows a Kaplan-Meier curve using the Kyoto samples (*n* = 499). *Each line* represents patients with the top 10% GRS values (*blue*), patients with lowest 10% GRS (*black*), and others (*red*). After 10 years from their first visit, patients with GRS values in the top 10% had a 51.0% hazard rate, in contrast to 2.3% among patients with GRS values in the lowest 10%. (**B**) The diagram shows a Kaplan-Meier curve using the Fukushima samples (*n* = 263). *Each line* represents patients with top 25% GRS values (*blue*), patients with lowest 25% GRS values (*black*), and others (*red*). After 10 years from their first visit, patients with GRS values in the top 10% had a 27.6% hazard rate, in contrast to the 1.7% rate among patients with GRS values in the lowest 10%.

patients are scarce. So far, several cross-sectional studies have reported the association of *CFH* and *ARMS2/HTRA1* with bilateral early or late AMD. For *CFH*, Despriet et al.[47] reported that patients homozygous for the Y402H variant had a higher OR for bilateral late AMD (OR, 17.93; 95% confidence interval [CI], 9.00–35.70) than unilateral late AMD (OR, 6.58; 95% CI, 3.47–12.48), while the Blue Mountain Eye Study and Los Angeles Latino Eye Study reported the association of *CFH* Y402H with bilateral involvement of early AMD, but not late

AMD.[33,35] In the study by Despriet et al.,[47] OR of bilateral involvement comparing AMD patients with risk homozygous at *CFH* Y402H to those with nonrisk homozygous was 17.93/6.58 = 2.72, which is relatively lower than the current HR of $1.77 \times 1.77 = 3.13$. For *ARMS2/HTRA1*, three groups, including the one that analyzed Age-Related Eye Disease Study (AREDS) data, showed its association with bilateral late AMD.[14,34,36] In contrast to *CFH*, previously reported ORs for *ARMS2/HTRA1*, though they varied from each other (i.e., ranging from 1.23 to
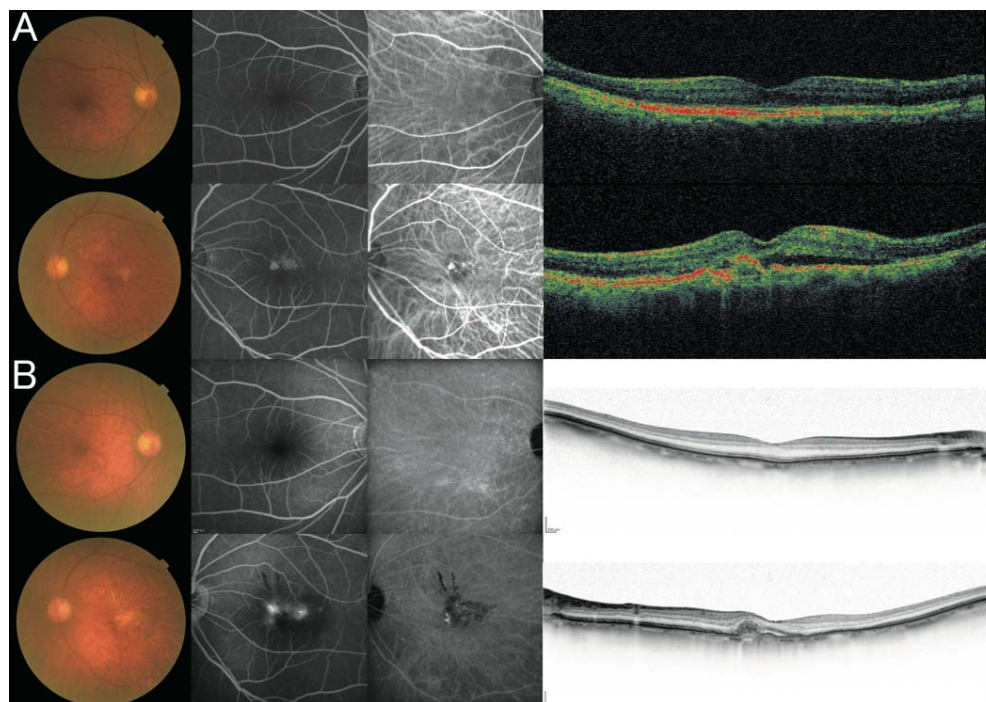

FIGURE 3. Color fundus photography (*first column*), fluorescein angiography (*second column*), indocyanine green angiography (*third column*), and optical coherence tomography (*fourth column*) images of a 78-year-old female whose genetic risk score was 0.21 (rs10490924, GT; rs800292, AA; rs943080, CC; rs79037040, TT; rs4698775, TT). (**A**) At the first visit, age-related macular degeneration was observed in her left eye, while no lesion was found in her right eye. Visual acuity was 20/20 in the right eye and 20/50 in the left eye. (**B**) Even after 7.7 years from her first visit, no lesion had been found in her right eye.
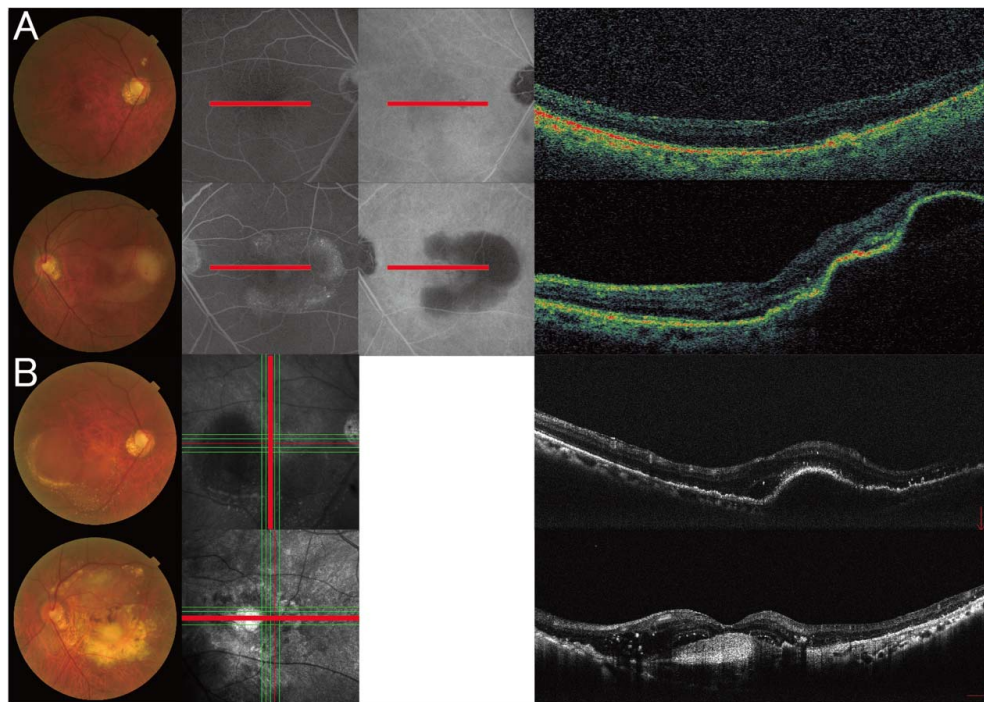
FIGURE 4. Images of 63-year-old male whose genetic risk score was 3.04 (rs10490924, TT; rs800292, GG; rs943080, CT; rs79037040, GG; rs4698775, GG). (**A**) Color fundus photography (*first column*), fluorescein angiography (*second column*), indocyanine green angiography (*third column*), and optical coherence tomography (*fourth column*) images are displayed. At the first visit, age-related macular degeneration (AMD) was observed in his left eye, while only pigment epithelium mottling was seen in his right eye. (**B**) Color fundus photography (*left column*), scanning laser ophthalmoscopy (*middle*), and optical coherence tomography (*right column*) images are displayed. Within 4.8 years from his first visit, exudative AMD developed in his fellow eye (right eye), presenting a large pigment epithelium detachment accompanied by moderate subretinal fluid.

2.43 for heterozygous and 2.01 to 4.84 for risk homozygous), are relatively higher than the current HR. We need to be careful regarding these under-/overestimates caused by *CFH* and *ARMS2/HTRA1* when we evaluate prevalence of bilateral AMD instead of incidence. In addition to these reports that evaluated prevalence of bilateral AMD, two reports have evaluated the association between AMD susceptibility genes and the incidence of bilateral AMD in unilateral late AMD patients. We previously conducted a retrospective longitudinal analysis of 207 unilateral AMD cases and reported that *ARMS2* was significantly associated with duration until second-eye involvement.[15] On the other hand, the CATT study group failed to show significant contribution of *CFH*, *ARMS2/HTRA1*, and *C3* to second-eye involvement by prospective follow-up of 518 unilateral AMD cases,[16] which is in contrast to many of the previous reports. Our study added further insights to this argument. Based on the current result showing that *ARMS2* and *CFH* were significantly associated with the duration until second-eye involvement in the meta-analysis of three longitudinal cohorts consisting of 891 unilateral AMD cases. The results of the CATT study might have been false negatives, possibly due to its relatively short follow-up period.

In addition to *ARMS2* and *CFH*, we confirmed consistent effects of *VEGFA*, *TNFRSF10A*, and *CFI* on second-eye involvement across three cohorts. The GRS values calculated from these five genes were significantly associated with second-eye involvement after adjustment for age and sex. Patients with low GRS values were less likely to develop AMD in their second eye for their age (Fig. 3), while patients with high GRS values tended to develop AMD in their second eye earlier for their age (Fig. 4). It is surprising that as many as 51.0% of patients with higher GRS values will develop AMD in their second eye within 10 years from their first visit. What is more important is the low hazard rate among patients with lower GRS values. These patients rarely develop AMD in their second eye, with a hazard rate within 10 years from their first visit ranging from 1.7% to 2.3%. Considering that patients with nonrisk homozygous *ARMS2* or *CFH* had a hazard rate of 10% to 20% (Figs. 1A, 1B), the predictive ability of GRS was considerably higher than that of any single SNP genotype. Though AMD is becoming controllable due to the progress of anti-VEGF treatment, its long-term prognosis is not necessarily good. Since bilateral involvement of AMD severely impairs patients' QOL, we might need to perform more intense treatment to maintain visual capacity of the first eye and frequent follow-up for the early detection of second-eye involvement, especially for the patients with high risk of second-eye involvement.

Although this study has strengths due to its relatively large sample size, repeated replication using three cohorts, and comprehensive evaluation of AMD susceptibility SNPs, it does also have limitations. First, it is retrospective. As most of the participants underwent treatment in each institute, the response to the treatment might be associated with loss to follow-up, which is a possible cause of selection bias. However, currently, it is controversial whether the AMD susceptibility genes are associated with the response to treatment. As such, we cannot assume that genetics are associated with loss to follow-up. Since noninformative selection does not cause bias, we cannot say that this retrospective study is biased by selection. Secondly, the follow-up intervals were different, which might have caused a delay in the diagnosis of the second-eye involvement. However, since second-eye involvement directly affects patient QOL, they would voluntarily visit the doctor if it occurs. Thus, delay in the diagnosis would not have affected the results much. Third, the current study did not

employ nongenetic factors, such as smoking status and macular status, within the model. Including these factors would further increase predictive ability. However, since evaluation of environmental factors was beyond the scope of this study, whose aim was to correlate genetic risk and second-eye involvement, this should be explored in the future studies. Lastly, the sample size is still a limitation. Though we included a relatively large number of patients, analysis using more samples would allow us to detect more susceptibility genes. It would also allow us to conduct reliable genome-wide survival analysis.

In conclusion, we demonstrated that AMD susceptibility genes were significantly associated with second-eye involvement using a large number of Japanese unilateral AMD patients across three independent longitudinal cohorts. The difference in hazard rates between patients with high GRS and low GRS values was considerable. By considering other environmental risk factors, the current approach may bring substantial benefits to real-world practice in the future.

Acknowledgments

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