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ARTICLE

Genome-wide association study suggests four variants influencing outcomes with ranibizumab therapy in exudative age-related macular degeneration

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Abstract

To identify factors associated with ranibizumab responses in patients with exudative age-related macular degeneration (AMD), we performed a genome-wide association study (GWAS) and a replication study using a total of 919 exudative AMD patients treated with intravitreal ranibizumab in a Japanese population. In the combined analysis of GWAS and the replication study, no loci reached genome-wide significant level; however, we found four variants showed suggestive level of associations with visual loss at month three (rs17822656, rs76150532, rs17296444, and rs75165563: $P_{\mathrm{combined}} < 1.0 \times 10^{-5}$). Of the candidate genes within these loci, three were relevant to VEGF-related pathway (*KCNMA1*, *SOCS2*, and *OTX2*). The proportions of patients who worsened visual acuity were 13.7%, 38.8%, 58.0%, and 80.0% in patients with 0, 1, 2, and 3 or more identified risk variants, respectively. Changes in visual acuity decreased linearly as the number of risk variants increased ($P = 1.67 \times 10^{-12}$). The area under the curve using age, baseline visual acuity, and history of previous treatment was 0.607, and improved significantly to 0.713 in combination with identified variants ($P < 0.0001$). Although further study is needed to confirm their associations, our results offer candidate variants influencing response to ranibizumab therapy.

Introduction

Age-related macular degeneration (AMD) is a chronic, progressive disease of the central retina in the elderly population and a leading cause of severe visual impairment worldwide [1, 2]. Treatment of exudative AMD changed dramatically with the introduction of anti-vascular endothelial growth factor (VEGF) agents. Previous randomized clinical trials showed that individuals treated with ranibizumab had improved best-corrected visual acuity (BCVA) at 12 months and maintained it for 24 months, whereas individuals treated with sham injection or photodynamic therapy (PDT) showed worsened BCVA [3, 4]. Moreover, a recent observational study reported that the incidence of blindness resulting from AMD has decreased since the induction of anti-VEGF treatment [5]. In contrast to these obvious benefits, anti-VEGF treatment has several problems, such as frequent follow-up and high medical costs [6]. In addition, 4–8% of patients reportedly show worsened visual acuity (loss of three or more lines on the visual acuity chart) after treatment [3, 4, 7]. Age, baseline visual acuity, and size of the choroidal neovascularization (CNV) are major clinical predictors of visual prognosis with anti-VEGF treatment [8–11], although clinical efficacy has not yet been clarified.

Recent advances in pharmacogenetic studies have enabled the identification of genetic markers associated with drug responses [12]. Most of the previous pharmacogenetic studies investigating the response to ocular anti-VEGF treatment examined single-nucleotide polymorphisms (SNPs) within AMD susceptibility loci or functional candidate genes; however, the results have been controversial

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[13–16]. So far, two genome-wide association studies (GWAS) have been reported. One was GWAS examined 65 patients in European population [17]. The other was recently reported one including a total of 461 Japanese individuals [18]. Nevertheless, these previous GWAS efforts could not detect loci significantly associated with ranibizumab response. Here, to identify predictive markers for the visual outcome of ranibizumab therapy, we conducted a GWAS and a replication study using a total of 919 exudative AMD patients in a Japanese population, which doubles the number of analyzed samples in comparison with that of the previous GWAS.

Materials and methods

Study population

We recruited 965 exudative AMD patients aged 50 years or older who underwent ranibizumab treatment. Written informed consent was obtained from all participants before the study. Approvals from ethics committees were obtained at all institutions.

Exudative AMD and its subtypes were diagnosed by comprehensive ophthalmic examinations, including ophthalmoscopic examination, fluorescein angiography, indocyanine green angiography, and optical coherence tomography (OCT) by specialized ophthalmologists in collaborating hospitals. All participants were treated with 0.5 mg ranibizumab under an as-needed regimen. At each hospital, re-injections were determined by ophthalmology specialists.

We excluded patients that met at least one of the following criteria: (1) ranibizumab was combined with other therapies (PDT, photocoagulation, ocular surgery, or ocular injection of steroid agent or gas) within 3 months; (2) the treated eye had a pathological myopia or angioid streaks; or (3) visual acuity was not quantified. Ultimately, we excluded 37 patients and included 928 patients for further analyses.

Samples included in the GWAS were collected from two facilities in the period until August 2012. Then, samples for the replication study were collected until June 2013 from seven facilities (Supplementary Note).

Data collection and phenotypic definition

We collected age, gender, baseline BCVA, AMD subtype, and previous treatments as baseline characteristics. We also collected BCVA, presence or absence of injections, and central retinal thickness every month from medical records. BCVA was measured using a Landolt chart, and converted into logarithm of the minimal angle of resolution (logMAR). Central retinal thickness was measured within the inner circle of 1 mm diameter using spectral domain OCT. For individuals in the replication study, we collected the same clinical information as the GWAS samples, but BCVA was only collected at baseline and 3 months. Angiographic features and central retinal thickness were not available in the replication study. Missing data were imputed using the last-observation-carrying-forward method.

For treatment outcome, we defined individuals whose BCVA worsened as non-responders, and those who maintained or improved their BCVA as responders, according to the change in BCVA at 3 months vs. baseline. Among the 37 patients who underwent bilateral treatment, we deemed the eye with the larger change in BCVA at 3 months as the study eye.

GWAS and replication study

The study design is shown in Supplementary Figure S1. For the GWAS, we genotyped 441 individuals using Illumina OmniExpress BeadChips. Among SNPs contained in the BeadChip, 553315 SNPs in the autosomal chromosomes passed quality control (QC) filters (call rate per SNP ≥ 0.99 and P for Hardy–Weinberg equilibrium in responders ≥ 1.0×10^{-6}). For stringent QC in the GWAS, we calculated identity by state, and excluded two closely related individuals. We performed principal component analysis [19], and excluded five outliers from further analyses (Supplementary Fig. S2). Finally, 434 individuals were included in the GWAS. Next, we performed whole-genome imputation using the 1000 Genomes project [20] data of the East Asian population as a reference, and 6826359 SNPs passed the QC filters (Supplementary Note).

Among 404 SNPs, which showed $P < 1.0 \times 10^{-4}$ after the imputation, we selected 116 independently associated SNPs that denoted a linkage disequilibrium (LD) coefficient of $r^2 \leq 0.6$. Among 116 SNPs, 57 SNPs were genotyped and 59 were imputed SNPs. Direct genotyping validated 36 of the 59 imputed SNPs with $P < 1.0 \times 10^{-4}$ by multiplex PCR-based Invader assay [21] (Supplementary Table S1). Finally, we selected 57 genotyped and 36 validated SNPs, and genotyped 487 individuals for the replication study. For the QC in the replication study, we excluded two individuals with a call rate < 0.95. After that, mean call rate was 99.9% (range: 99.4–100%) and P values for HWE test were not deviated (lowest P value for HWE $= 2.48 \times 10^{-3}$) in the replication set.

To evaluate concordance between the BeadChip and Invader assays, we randomly genotyped 15 SNPs on the BeadChip by the Invader assay for all GWAS samples, and observed perfect concordance between the two platforms.

Table 1 Baseline characteristics of study subjects

Characteristics	GWAS		Replication study	
	Non-responder ($n = 73$)	Responder ($n = 361$)	Non-responder ($n = 122$)	Responder ($n = 363$)
Sex no. (%)				
Male	47 (64.4)	237 (65.7)	87 (71.3)	254 (70.0)
Female	26 (35.6)	124 (34.3)	35 (28.7)	109 (30.0)
Age yr[a]				
Mean	75.3 ± 7.9	73.6 ± 8.3	73.5 ± 8.3	72.9 ± 8.4
Range	54–91	51–93	52–89	50–90
Age group no. (%)				
< 65 yr	7 (9.6)	54 (15.0)	16 (13.1)	66 (18.2)
65–74 yr	25 (34.2)	127 (35.2)	49 (40.2)	128 (35.3)
75–84 yr	34 (46.6)	154 (42.7)	44 (36.1)	143 (39.4)
≥ 85 yr	7 (9.6)	26 (7.2)	13 (10.7)	26 (7.2)
Visual acuity[b]				
20/200 or worse	16 (21.9)	70 (19.4)	13 (10.7)	45 (12.4)
Better than 20/160 but worse than 20/50	19 (26.0)	116 (32.1)	31 (25.4)	98 (27.0)
Better than or equal to 20/50 but worse than 20/25	28 (38.4)	142 (39.3)	45 (36.9)	141 (38.8)
20/25 or better	10 (13.7)	33 (9.1)	33 (27.0)	79 (21.8)
Central retinal thickness (μm)[c,d]	355.7 ± 122.1	357.8 ± 117.7	NA	NA
Subtypes no. (%)[e]				
Typical age-related macular degeneration	39 (54.2)	207 (57.3)	50 (41.0)	173 (47.7)
Predominantly classic choroidal neovascularization	15 (38.5)	55 (26.6)	NA	NA
Minimally classic choroidal neovascularization	7 (17.9)	56 (27.1)	NA	NA
Occult choroidal neovascularization	17 (43.6)	95 (45.9)	NA	NA
Missing data	0	1 (0.5)	NA	NA
Polypoidal choroidal vasculopathy (PCV)	32 (43.8)	142 (39.3)	66 (54.1)	174 (47.9)
Retinal angiomatous proliferation	2 (2.7)	11 (3.0)	5 (4.1)	15 (4.1)
Choroidal neovascularization and PCV in same eye	0	0	0	1 (0.3)
Unclassified	0	1 (0.3)	1 (0.8)	0
Treatment-naive no. (%)[f]	54 (74.0)	309 (85.6)[g]	89 (73.6)	302 (83.2)[g]
Previous therapy no. (%)				
Laser photocoagulation	2 (10.5)	5 (9.6)	1 (3.1)	4 (6.6)
Photodynamic therapy (PDT)	9 (52.9)	34 (72.3)	22 (68.8)	46 (75.4)
PDT + anti-VEGF agent combined therapy	2 (10.5)	5 (9.6)	3 (9.4)	3 (4.9)
previous use of anti-VEGF agent monotherapy	3 (15.8)	5 (9.6)	4 (12.5)	5 (8.2)
Other[h]	3 (15.8)	3 (5.8)	2 (6.3)	3 (4.9)

[a]Plus-minus values are means ± standard deviations

[b]Best-corrected decimal visual acuity obtained by Landolt ring chart were converted into fractional visual acuity

[c]Central retinal thickness were measured within the inner circle of 1 mm diameter using spectral domain optical coherence tomography (OCT)

[d]Data missing for seven patients in the genome-wide association study (GWAS)

[e]Subtypes were diagnosed based on its characteristics of fundus, angiographic characteristics, and OCT feature

[f]Data missing for one patient in the replication study

[g]Proportion of the responder with treatment-naive was significantly higher compared with that of non-responder ($P < 0.05$)

[h]Other therapy include vitreous surgery, transpapillary thermo therapy, radiation, intraocular injection of tissue plasminogen activator and gas, subtenon triamcinoron acetonide injection, and rapamycin

GWAS = genome-wide association study; PCV = polypoidal choroidal vasculopathy; VEGF = vascular endothelial growth factor

Statistical analysis

The association tests for directly genotyped SNPs were performed by a one-degree-of-freedom Cochran-Armitage trend test. The odds ratio (OR) and its confidence interval (CI) were calculated from a 2 × 2 contingency table using risk alleles for non-responders in the GWAS as a reference. Association tests in whole-genome imputation were calculated for using allele dosage by mach2dat [22]. We set the significant threshold in the replication study as $P < 5.38 \times$

10^{-4} using Bonferroni correction. We combined the results from the GWAS and the replication study using the Mantel-Haenszal method. Heterogeneity across the study was examined using the Breslow-Day test.

We examined the relationship between the number of risk alleles among four SNPs, and the quantitative change in BCVA using linear regression analysis. Two individuals who missed one of the four genotypes were excluded. Individuals who had three or four risk alleles were combined into one category because of the small number of samples. We analyzed GWAS and replication samples separately, and combined the results using the inverse-variance method. Heterogeneity was examined using the Cochran's Q test.

To estimate the impact of genetic and clinical parameters on ranibizumab response, we constructed risk score models and estimated using receiver operation characteristic (ROC) analyses. We selected four candidate SNPs and clinical parameters (age, gender, baseline BCVA, previous treatment, subtypes of exudative AMD). Multivariate stepwise logistic regression analysis was performed using clinical parameters only, genetic parameters only, and both parameters. We excluded five individuals who were missing one of the parameters, and set the P value threshold to < 0.1 in the stepwise procedure. Risk score was generated by substitution of the β-coefficient of the final models. We generated ROC curves in three models and evaluated areas under the curves (AUCs). The differences in AUC among the models were calculated using a bootstrap resampling method with 10,000 replicates.

We estimated statistical power to detect genome-wide significant level of association in the analyzed sample size ($N = 919$) by a one-degree-of-freedom Cochran-Armitage trend test by permutation procedure with 100,000 replicates using R.

We used the 'R' software (ver. 2.15.3 and 3.3.3) for statistical testing. Haploview was used to analyze LD values [23]. Bootstrap resampling was performed using the pROC package [24]. Regional association plots were generated using LocusZoom [25].

Results

Phenotype definition

We classified individuals based on the change in BCVA at 3 months, and 73 of 434 (16.8%) participants in the GWAS and 122 of 485 (28.1%) participants in the replication study were deemed non-responders. Baseline characteristics were not statistically different between responders and non-responders, except in the proportion of patients who were treatment-naive (Tables 1, 84.4% in responders and 73.0% in non-responders; $P = 0.001$, OR = 1.93, 95% CI = 1.29–2.85). When we examined the change in BCVA for 12 months using 367 participants followed over 12 months without other treatment, BCVA improved linearly up to 3 months, and was maintained for 12 months in responders. In contrast, BCVA declined rapidly up to 2 months, and did not improve over 12 months in non-responders. We observed significant differences in change in BCVA between responders and non-responders for all periods, from 1–12 months ($P < 0.0001$; Fig. 1).

GWAS and replication study

We performed a GWAS, which included 73 non-responders and 361 responders. The genomic inflation factor was 1.014 (Supplementary Fig. S2), indicating a low possibility of false-positive associations resulting from population stratification. After whole-genome imputation and validation for highly associated SNPs, we genotyped 93 SNPs successfully using an independent set of 485 samples in the replication study. Although we did not find significant SNPs in the replication study ($\alpha = 5.38 \times 10^{-4}$), four SNPs on chromosome 3q12, 10q22, 12q22, and 14q22 surpassed the suggestive level of association ($P < 1.0 \times 10^{-5}$) after combining the results of the GWAS and the replication study (Table 2, Supplementary Fig. S3). Risk allele frequencies of these SNPs were low in responders (2.6–5.0%), but more than twofold higher in non-responders (8.3–11.5%).

Cumulative effect of identified variants

We calculated the number of candidate risk alleles for participants, and examined the cumulative effect on treatment response (Supplementary Table S2). Proportions of responders decreased as number of risk alleles increased (Supplementary Fig. S4). Notably, 37 of 60 (61.7%) participants who had two or more risk alleles were non-responders. When we examined the association for cumulative risk alleles with change in BCVA at 3 months, we observed significant linear trends in both the GWAS ($P = 9.56 \times 10^{-9}$) and the replication study ($P = 2.93 \times 10^{-5}$). The combined result also suggested a significant linear trend ($P_{combined} = 1.67 \times 10^{-12}$, Fig. 2). The burden of a risk allele for change in BCVA was estimated as + 0.09 logMAR per category. The difference in linear trend across the studies was not statistically significant ($P_{het} = 0.14$).

Risk score model using genetic and clinical predictors

To investigate the impact of predictive factors on treatment response, we constructed risk score models using clinical factors only (age, baseline BCVA, previous treatment), genetic factors only, and both factors (Supplementary

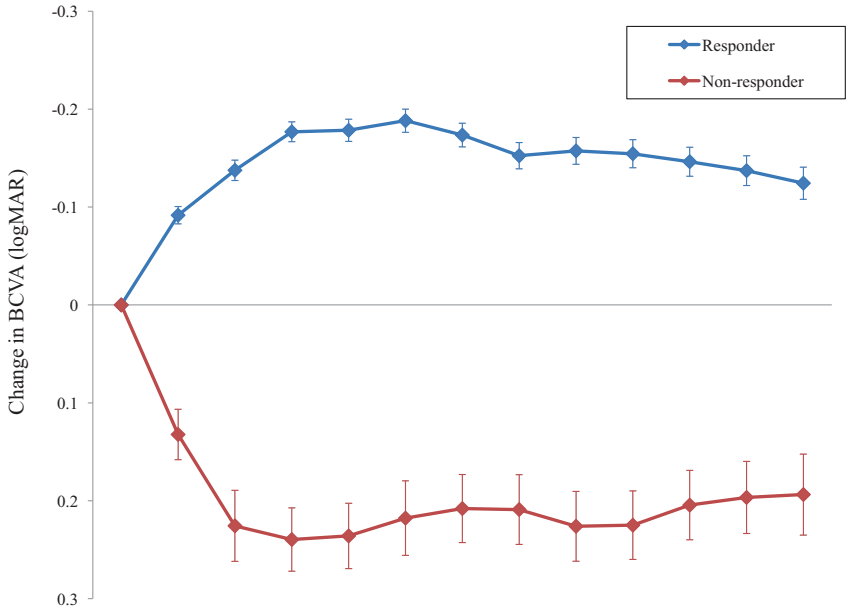

Fig. 1 Change in visual acuity during a 12-month period between responders and non-responders. Visual acuity changes in 367 individuals (303 responders, 64 non-responders) among genome-wide association study samples are shown. Patients who were observed for > 1 year without other treatment were included in the analysis. Missing data were imputed by last-observed-carried-forward method. Differences in change in best-corrected visual acuity (BCVA) between responders and non-responders were significant ($P < 0.0001$) from 1 to 12 months. Mean and standard error of logarithm of the minimal angle of resolution (logMAR) change in BCVA are shown in the table. BCVA = best-corrected visual acuity; logMAR = logarithm of the minimal angle of resolution; SE = standard error

Change in BCVA from baseline		1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M	12M
Responder	Mean	-0.09	-0.14	-0.18	-0.18	-0.19	-0.17	-0.15	-0.16	-0.15	-0.15	-0.14	-0.12
	SE	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)
Non-responder	Mean	+0.13	+0.23	+0.24	+0.24	+0.22	+0.21	+0.21	+0.23	+0.22	+0.20	+0.20	+0.19
	SE	(0.03)	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)	(0.04)	(0.04)	(0.03)	(0.04)	(0.04)	(0.04)

Table S3), and generated ROC curves (Fig. 3). AUCs were 0.607 (95% CI = 0.559–0.654) and 0.670 (95% CI = 0.629–0.712) for clinical and genetic factors only, respectively. The combined model using clinical and genetic parameters showed an AUC of 0.713 (95% CI = 0.670–0.755), and significantly improved vs. that of the clinical model ($P < 0.0001$).

Assessment of confounders

To assess possible confounders influencing the association of four candidate SNPs, we performed a conditional analysis and a stratified analysis using 914 individuals who were included in the risk score analysis. First, we evaluated the associations of four candidate SNPs by adjusting for the number of ranibizumab injections, and observed similar effect sizes between those of crude and adjusted for number of injections (Supplementary Table S4). Second, we subdivided analyzed individuals into typical neovascular AMD and PCV, and carried out stratified analysis. However, we did not observe significant differences between them (Supplementary Table S5).

Discussion

We performed GWAS and a replication study for visual outcomes with ranibizumab therapy using 919 exudative AMD patients in a Japanese population. Although we could not detect variants satisfying genome-wide significance, four low-frequency variants that showed suggestive levels of associations ($P < 1.0 \times 10^{-5}$) were found. Accumulation of these SNPs strongly influenced the proportion of patients with visual loss and the decline in BCVA at 3 months. A risk score model constructed with age, baseline BCVA, previous treatment, and the four candidate SNPs showed moderate predictive accuracy (AUC > 0.7) for ranibizumab response. Considering genetic parameters significantly improved the predictive accuracy, genetic factors may contribute to visual outcome with ranibizumab therapy.

Unlike the candidate gene approach, GWAS is a hypothesis-free approach. We investigated involvement of positional candidate genes of four suggested loci in VEGF-related pathways using Ingenuity Pathway Analysis software (Supplementary Note). Among the positional candidate genes, three genes (*KCNMA1*, *SOCS2*, and *OTX2*) located at independent loci were found to be involved with genes belonging to VEGF-related pathways (Supplementary Fig.S5). Moreover, higher expression of *KCNMA1* is reported to be associated with steroid-induced intraocular pressure elevation in bovine trabecular meshwork [26], indicating that *KCNMA1*, which affects outflow resistance of the aqueous humor, may change intraocular drug clearance. As another candidate gene of interest, *CRADD* is known to induce apoptosis [27], and may influence visual

Table 2 Result of genome-wide association study and replication study

SNP	Chr. position[a] [risk/non-risk][b]	Nearest gene	Stage	Non-responder				Responder				*P* value	OR	95% CI	*P* het
				RR	RN	NN	RAF	RR	RN	NN	RAF				
rs75165563	14q22	*EXOC5*	GWAS	0	15	58	0.103	1	15	345	0.024	4.85×10^{-6}	4.75	(2.71–8.33)	
	57,687,646		Replication	1	15	106	0.070	0	21	342	0.029	0.005	2.51	(1.44–4.39)	
	[C/A]		Combine									7.49×10^{-7}	3.28	(2.02–5.33)	0.20
rs76150532	10q22	*KCNMA1*	GWAS	1	20	52	0.151	0	28	332	0.039	9.76×10^{-8}	4.38	(1.65–11.67)	
	79,130,974		Replication	2	17	103	0.086	2	33	328	0.051	0.05	1.75	(0.90–3.43)	
	[A/G]		Combine									2.13×10^{-6}	2.60	(1.74–3.88)	0.03
rs17822656	3q12	*ZPLD1*	GWAS	1	16	56	0.123	1	26	334	0.039	4.42×10^{-5}	3.49	(2.08–5.84)	
	102,189,169		Replication	0	24	98	0.098	1	32	329	0.047	0.003	2.21	(1.38–3.56)	
	[G/A]		Combine									2.27×10^{-6}	2.66	(1.76–4.00)	0.28
rs17296444	12q22	*PLXNC1*	GWAS	1	18	54	0.137	0	33	328	0.046	2.10×10^{-5}	3.31	(1.35–8.11)	
	94,450,572		Replication	1	23	98	0.102	1	38	324	0.055	0.01	1.96	(1.02–3.76)	
	[T/C]		Combine									7.32×10^{-6}	2.43	(1.64–3.59)	0.19

[a]Chromosomal position based on NCBI human genome Build 37 coordinates

[b]Risk allele was defined as allele showed higher frequency in poor responder in GWAS

SNP single-nucleotide polymorphism, *Chr.* chromosome, *RR* risk allele homozygote, *RN* risk allele heterozygote, *NN* non-risk allele homozygote, *RAF* risk allele frequency, *OR* odds ratio, *CI* confidence interval

prognosis by affecting retinal cell survival. All of these candidate genes are expressed in the human retina and cultured retinal pigment epithelium cells, according to the Human Retinal Transcriptome Browser [28] and GEO database (GSE17938) [29]. Our findings may be expected to provide novel insights into the mechanisms of ranibizumab action and the development of new therapeutic targets [12].

Our results suggested the cumulative effects of the SNPs identified on visual outcomes. These SNPs were independently associated, even after adjustment for other SNPs and clinical predictors (Supplementary Table S3). Although the frequency of each SNP was low, 326 of 917 (35.6%) of our participants had at least one risk allele. This implies that some patients were genetically susceptible to worsened visual function, even when treated with ranibizumab, especially patients with several risk alleles.

To the best of our knowledge, this is the first reported evaluation of predictive factors including both clinical and genetic factors for ranibizumab responses. In addition to genetic factors, we demonstrated that a history of previous treatment was a significant predictive factor for visual loss. Indeed, its impact has not been well investigated, because recent clinical trials tended to exclude patients with a history of previous treatment [4, 7]. Nevertheless, it would be possible that disease duration might be shorter in individuals of treatment-naive and influence the difference in visual outcomes. Although our model reached moderate predictive accuracy, the model should be studied in other populations to avoid overfitting.

The strength of this study was including a large number of patients with AMD who were treated by ranibizumab. For example, the previously reported large-scale pharmacogenetics study in Asian, which was conducted by candidate gene approach, investigated the genetic associations of 17 SNPs in 394 patients with exudative AMD in Korean [18]. As for GWAS, Yamashiro et al. reported GWAS in 256 Japanese individuals with exudative AMD and replication study included 205 independent samples. In comparison with the previous studies conducted in Asia, the number of samples analyzed in the current study was more than twice.

Our study had several limitations. First, we determined ranibizumab responses according to change in BCVA at 3 months. This period might be too early to predict the individual's responses. However, when we analyzed the change in BCVA from baseline in 367 participants analyzed in Fig. 1, changes in BCVA were significantly correlated between at 3 months and at 12 months (Pearson's correlation coefficient = 0.67, 95%CI = 0.61–0.72, $P < 0.0001$). In addition, the proportion of patients who worsened BCVA at 12 months vs. baseline were significantly higher in non-responders compared with that of responders ($P < 0.0001$,

a

Change in BCVA at month theree (logMAR)	Number of risk alleles				Linear regression			p_{het}
	0	1	2	3 or 4	p value	*B*	SE	
GWAS	-0.14±0.19	-0.05±0.30	+0.11±0.39	+0.11±0.05	9.56×10^{-9}	0.11	0.02	
Replication study	-0.09±0.23	-0.02±0.26	+0.07±0.27	+0.08±0.09	2.93×10^{-5}	0.07	0.02	
Combine					1.67×10^{-12}	0.09	0.01	0.14

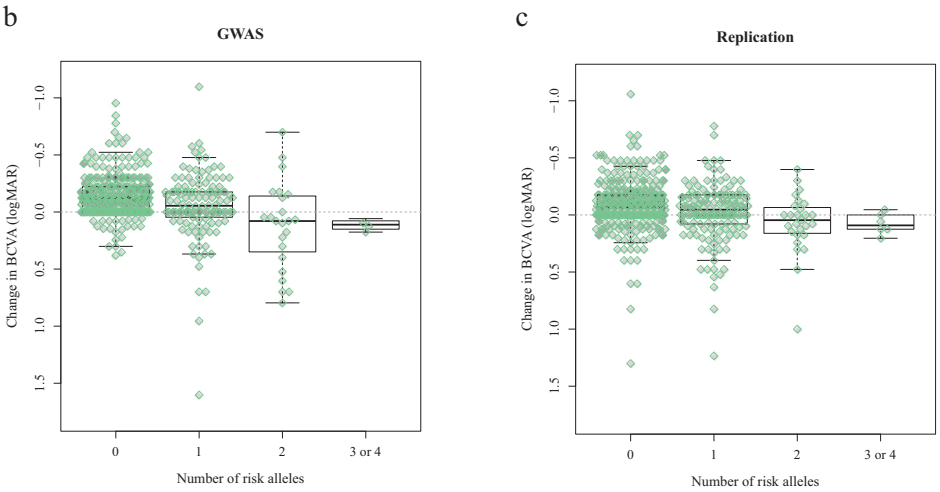

Fig. 2 Accumulation of risk alleles decrease visual acuity at 3 months. We assessed the association between numbers of risk alleles with change in best-corrected visual acuity (BCVA) using linear regression analysis **a**. Plus-minus values are means ± standard deviations of logarithm of the minimal angle of resolution (logMAR) change in BCVA at 3 months. Changes in logMAR BCVA were plotted by individuals and as boxplots for the genome-wide association study **b** and replication study **c**, respectively. Individuals missing one of the four genotypes were excluded from the analysis. BCVA = best-corrected visual acuity; logMAR = logarithm of the minimal angle of resolution; SE = standard error; GWAS = genome-wide association study

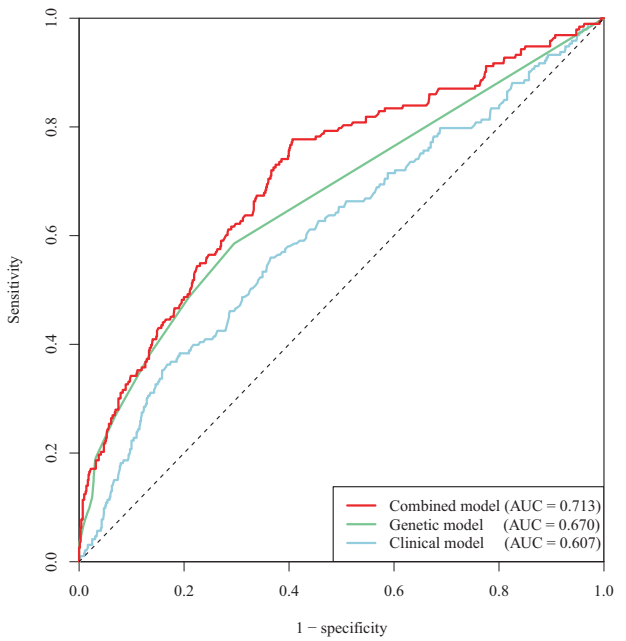

Fig. 3 Receiver operating characteristic curves for the ranibizumab response in clinical, genetic, and combined risk score models. We plotted receiver operating characteristic (ROC) curves for ranibizumab response using three models. ROC = receiver operating characteristic; AUC = area under the curve

OR = 7.14, 95% CI = 3.86–13.47). Indeed, 81.4% of the responders at 3 months maintained their visual acuity at 12 months. Although our phenotype definition was specialized for relatively early responses, these results suggest our findings might be applicable to later treatment outcomes. Second, although it is the largest pharmacogenetic study for ranibizumab response reported to date, candidate SNPs did not surpass the genome-wide level of association. In our power calculation, analyzed sample size had the statistical power of ≥ 80% to detect variants with OR ≥ 2.5 and risk allele frequency ≥ 15% at a genome-wide significant level ($P = 5.0 \times 10^{-8}$; Supplementary Table S6). Further studies are needed to validate our findings. Third, some baseline characteristics and treatment outcomes differed between the GWAS and replication study (Supplementary Table S7). Although we collected study participants serially, these differences induced sampling bias. Indeed, effect sizes of four candidate SNPs in the replication study were smaller than those of GWAS. Therefore, it would be possible that sampling bias weaken the associations of identified SNPs in the replication study. Fourth, we missed some clinical predictors, such as the features of CNV and OCT [11]. Including these predictors may improve the predictive accuracy of our risk score models. Fifth, our study focused only on visual acuity as a clinical outcome. Assessing retinal anatomical changes which can be evaluated by OCT will also provide insights into the genetic components associated with response to ranibizumab therapy. Further study with large sample size and more detailed clinical information is needed to verify our findings.

In conclusion, we showed four low-frequency variants, which potentially influence visual outcomes with ranibizumab therapy in patients with exudative AMD. Although further validation is needed, these variants may make it possible to improve the prediction of responses to ranibizumab therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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