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Original Article

Pharmacogenetics of genes associated with outcome of conbercept treatment for age-related macular degeneration in a Chinese population

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Abstract: To ascertain whether single-nucleotide polymorphisms (SNP) of complement factor H (*CFH*), interleukin 8 (*IL8*), vascular endothelial growth factor A (*VEGFA*), age-related maculopathy susceptibility 2 (*LOC387715/ARMS2*), high-temperature requirement A-1 (*HTRA1*), and complement component C3 (*C3*) genes impact the outcomes of neovascular age-related macular degeneration (AMD) by conbercept in Chinese patients, we enrolled 184 neovascular AMD patients from the AURORA and PHOENIX trials, and participants were administrated conbercept. For each patient, baseline best corrected visual acuity (BCVA) letter score was recorded, and the BCVA score at each subsequent visit, age, sex, and the number of injections were also recorded. Seven candidate SNPs were selected. The SNP genotyping was performed by TaqMan SNP genotyping assays. Single-locus analysis and haplotype analysis were used to determine the influence of each SNP on treatment outcome at 6 and 12 months. We found that, both in single-locus analysis and haplotype analysis, *rs10490924* and *rs11200638* were significantly associated with patient response to conbercept treatment for neovascular AMD in the Chinese population. These findings suggest that SNPs *rs10490924* and *rs11200638* could be used as genetic biomarkers for estimation of visual outcomes in response to conbercept treatment for neovascular AMD. As a result, a subgroup of Chinese patients that benefit from this modality of treatment can be identified.

Keywords: SNP, neovascular AMD, conbercept, SNP genotyping, genetic biomarker

Introduction

Age-related macular degeneration (AMD) is a common eye condition and a leading cause of vision loss among people aged 50 and older, which makes up 8.7% of all cases of blindness all over the world [1]. In its advanced stages, the disease has two forms, neovascular ("wet") AMD involving the development of choroidal neovascularization (CNV) and geographic atrophy ("late dry") characterized by atrophic changes in the central macula [2]. The neovascular (wet or exudative) type of AMD accounts for only 10-15% of AMD cases, but accounts for up to 90% of severe visual loss in consequence of AMD [3]. Although the pathophysiology of neovascular AMD remains largely unknown, it is proven that vascular endothelial growth factor (VEGF) is one of the major factors that impacts the process of neovascularization and angiogenesis [4, 5]. In fact, anti-VEGF agents are now widely used for treating neovascular AMD. Most VEGF inhibitors in clinical use are proteins, and a few are small molecules [6]. Peptide agents can be effective supplementary parts to the existing drugs [7].

Intra-vitreal administration of anti-VEGF drugs, including pegaptanib (Macugen; Eyetech Pharmaceuticals, Lexington, MA) [8], ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA) [9], bevacizumab (Avastin; Genentech and

Roche, Basel, Switzerland) [10], aflibercept (Eylea; Regeneron, Tarrytown, NY; and Bayer, Leverkusen, Germany) [11], and conbercept (KH902; Chengdu Kanghong Biotech Co., Ltd., Sichuan, China) [12-18], has become the mainstay of current treatment for neovascular AMD. Conbercept, also known as KH902, is a new and effective anti-VEGF agent, belonging to the new generation of anti-VEGF agents, as well as aflibercept. Although most neovascular AMD patients in the AURORA and PHOENIX trials benefited from conbercept treatment, we found that the treatment was ineffective in a few patients. Genetic background may be one factor causing the variations. There are currently no pharmacogenomics studies on the new generation of anti-VEGF drugs.

Previous studies have revealed that variants of the complement factor H (*CFH*) gene, interleukin 8 (*IL8*) gene, vascular endothelial growth factor (*VEGF*) A gene, age-related maculopathy susceptibility 2 (*LOC387715/ARMS2*) gene, high-temperature requirement A-1 (*HTRA1*) gene, and complement component C3 (*C3*) gene are associated with treatment outcomes of ranibizumab or bevacizumab treatment [19-22]. Furthermore, it has been reported that certain known susceptibility variants for AMD are correlated with anti-VEGF drug response [23]. East Asian populations have common variants increasing risk for AMD with populations of European ancestry, but they also hold specific genetic loci conferring AMD susceptibility [24]. It is ambiguous whether there are differences in the correlation of variants with response to anti-VEGF treatment between Asian patients versus patients of Europe. Thus, it is of significance to discover potential associations between genetic variants and efficacy of anti-VEGF therapy for neovascular AMD in the Chinese population.

In this report, we selected 7 single nucleotide polymorphisms (SNPs) within these genes to clarify whether the variants within these genes have an effect on the treatment with conbercept in the Chinese population. We conducted both single-locus analysis and haplotype analysis. These analyses demonstrated that *rs10490924* and *rs11200638* were significantly associated with response to treatment with conbercept at 6 months and 12 months. Patients carrying the *GG* or *GA* genotype for *HTRA1 rs11200638* and the *GG* or *GT* genotype for *LOC387715/ARMS2 rs10490924* were more likely to be responders compared with *AA* or *TT* genotypes carriers. These findings suggest that an effective and reasonable therapeutic plan with conbercept can be formulated if we have knowledge of genetic variations of AMD patients.

Methods

Study design and patient selection

All patients in this study were from the AURORA and PHOENIX trials. Study protocol and procedures for AURORA had been previously reported [17], and are available at ClinicalTrials.gov (NCT01157715). The PHOENIX trial is registered at ClinicalTrials.gov as NCT01436864. Before the study, we obtained written informed consent from all participants involved in the genetics ancillary study. In total, 184 consecutive patients (79, AURORA; 105, PHOENIX) were enrolled. Only one eye of each patient was included in the study. Relevant institutional review boards and ethics committees from the respective study centers approved the research protocols and its amendments. Research was performed in accordance with the tenets of the Declaration of Helsinki.

Inclusion and exclusion criteria

Eligibility criteria were as follows: (1) An age of 50 years or older; (2) The presence of untreated active subfoveal or juxtafoveal CNV secondary to AMD in the study eye; (3) Both eyes with lesion size of 12 disc areas or less and the BCVA score of the study eye between 73 and 19 letters [17]. The BCVA score was measured with the Early Treatment Diabetic Retinopathy Study chart (4-m starting distance). The exclusion criteria included: presence of CNV secondary to non-AMD conditions in either eye; previous drug treatment for AMD before conbercept administration; history of either laser therapy or other ocular operation in the study eye; presence of any serious adverse events during the course of treatment; or previous allergy to povidone iodine or fluorescein [17].

Treatment

Patients were intra-vitreally administrated a dose of 0.5 mg or 2.0 mg conbercept. In detail, all patients from PHOENIX received 0.5 mg treatment. In the AURORA trial, 42 out of 79

participants received 2.0 mg conbercept, and the rest were treated with 0.5 mg. Our previous study has demonstrated that there is no significant difference between the two dosing groups with respect to changes in the lesions [17]. In addition, we carried out a two-sample *t*-test with our data in this study, and found that there were no significant difference in treatment outcome between these two groups (p = 0.686). All patients from AURORA received 3 initial monthly conbercept injections. After 3 months' treatment, some patients were retreated at each subsequent visit according to retreatment criteria [17], the remaining patients received conbercept treatment monthly without changing the drug dose. For patients from PHOENIX, a part of patients received intra-vitreal injection of conbercept once per month for three times in the study eye, and then they received 2 sham injections monthly. Following these injections, they received conbercept treatment once every three months until month 12. However, 36 PHOENIX patients received sham injection monthly during the initial three months and then they received intravitreal injection of conbercept monthly three times, and thereafter they received conbercept therapy once every three months.

Measures of treatment response

The clinical indicator of treatment outcome was the mean change in BCVA from baseline. All examiners and readers were blinded to the treatment assignment. According to the mean change in BCVA from baseline at 6 months, we defined the response level by using a margin of 5 letters, which is the same to the study protocol of Abedi and colleagues [9]. In our study, patients with a gain of > 5 Early Treatment Diabetic Retinopathy Study (ETDRS) letters from baseline at 6 months were defined as responders, and those who gained ≤5 letters were designated as non-responders. We also classified the patients based on the mean change in BCVA from baseline at 12 months using the above criteria. However, 36 of 184 patients were administered with a sham injection during the first three months. To exclude the impact on the subsequent analysis, we utilized the X^2 test to determine the ratio of such patients among the responders and non-responders. The results revealed that the ratio was not statistically significant with *P* values of 0.92 and 0.33 at 6 months and 12 months, respectively.

DNA preparation and genetic analysis

Blood samples were collected from patients participating in the Aurora and Phoenix studies. Approximately 10 ml of peripheral blood was obtained from each patient at initial presentation. Genomic DNA was prepared using QIAamp DNA Blood Mini Kit by QIAGEN (Germantown, MD, USA). Seven candidate SNPs in the *CFH*, *VEGFA*, *ASMS2*, *HTRA1*, *IL8,* and *C3* genes were selected. All genetic variants were genotyped using the real-time-PCR-based TaqMan SNP genotyping assays by Applied Biosystems Inc. (Foster City, CA, USA) according to the manufacturer's instructions. Custom TaqMan SNP genotyping assays were designed for *rs13-29428*, *rs2227306*, *rs3025039*, *rs10490924*, *rs11200638,* and *rs2250656*. Pre-designed TaqMan SNP genotyping assay was for *rs1061170*.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for genotype distributions in both groups was evaluated using Fisher's exact test. The non-genetic covariates, including age, gender, baseline BCVA and the number of injections were used in the analysis. Descriptive statistics for all variables were calculated. Analysis of variance (ANOVA) was used to determine the influence of non-genetic covariates on continuous outcome variables (i.e., mean change in BCVA). Comparisons were made using the Chi-square test for categorical data (e.g. gender). All these tests were two-side test. Statistical analyses were performed by using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL).

The genotype distributions of selected SNPs among responders and non-responders were compared by using a Chi square test with 2 degrees of freedom (df). The association between genotype and visual response after 6 months and 12 months was evaluated using logistic regression analyses. For each genetic variant, the analysis was performed independent of other variants using the dominant genetic model with adjustment for age, gender, and baseline BCVA. The Bonferroni method was used to correct for multiple testing. A *P* value of 0.008 (= 0.05/6) was considered statistically significant after Bonferroni correction. SNP association analyses were performed using PLINK software version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) [25].

Table 1. Characteristics of candidate genetic markers in this cohort at 6 months

dbSNP ID	Gene	Chr	Minor/Major Allele	MAF	HWE *P*-value	
					Responders	Non-responders
rs1329428	CFH	1	T/C	0.33	0.84	0.77
rs1061170	CFH	1	C/T	0.10	1.0	1
rs2227306	IL8	4	T/C	0.33	0.68	0.57
rs3025039	VEGFA	6	T/C	0.17	1.0	< 0.05
rs10490924	ARMS2	10	G/T	0.32	0.24	0.31
rs11200638	HTRA1	10	G/A	0.33	0.17	0.28
rs2250656	C3	19	C/T	0.23	0.79	0.73

Note: SNP, Single nucleotide polymorphism (dbSNP ID); Chr, Chromosome; MAF, Minor allele frequency; HWE, Hardy-Weinberg Equilibrium. Significance accepted at $p < 0.05$.

Table 2. Comparisons of clinical characteristics of responders and non-responders at 6 months

Variables	Overall	Responders	Non-responders	*P* value[a]
Number of Patients	184	116	68	-
Gender, Male/Female	123/61	80/36	43/25	0.43
Age, mean ± SD	65.42 ± 8.05	64.84 ± 8.08	66.41 ± 7.95	0.20
Number of injections	5.18 ± 0.64	5.15 ± 0.62	5.23 ± 0.67	0.37
BCVA, mean ± SD				
At baseline	49.96 ± 15.04	50.24 ± 14.83	49.49 ± 15.48	0.74
After 6 months	60.09 ± 18.25	67.11 ± 15.99	48.12 ± 15.51	0.00*
Change at 6 months	10.13 ± 11.81	16.87 ± 8.17	-1.37 ± 7.32	0.00*

Note: [a]Two-sided Chi square test and ANOVA test were used to compare clinical characteristics between the responders and non-responders. SD, standard deviation; BCVA, best corrected visual acuity. *Both the difference in the mean change in BCVA from baseline at 6 months and the difference in the BCVA measured at 6 months between the responders and non-responders are statistically significant ($p < 0.05$).

Linkage disequilibrium (LD) calculation and haplotype blocks were estimated under the default settings of Haploview with use of data from all patients [26]. We used the parameter r^2 to describe the magnitude of LD. The R package *haplo.stats* was utilized to assess the association between treatment outcome and haplotypes. Both global tests and haplotype-specific tests were performed under the dominant effect. *P*-values smaller than 0.05 were considered statistically significant.

Results

Characteristics and genotyping results for candidate SNPs

The characteristics and overall genotyping results for candidate polymorphisms at 6 months and 12 months are listed in **Table 1** and Table S1 (available at http://www.aaojournal.org), respectively. The genotyping rate for all SNPs is 100%. Hardy-Weinberg equilibrium for genotype distributions was checked by Fisher's exact test. The distribution of genotypes for each genetic variant was consistent with the HWE ($p > 0.05$), except for SNP *rs3025039* ($p < 0.05$). Therefore, this variant was not involved in the following analyses.

Characteristics of patient cohort and treatment outcome

On the whole, 184 neovascular AMD patients were recruited. Clinical characteristics of the participants at 6 months were described in **Table 2**, including baseline BCVA, BCVA measured at 6 months, BCVA change from baseline at 6 months, age and gender distribution. The mean age of the patients was 65.42 ± 8.05 years, and the mean BCVA at baseline was 49.96 ± 15.04 ETDRS letters. More male patients (123, 67%) were included in this cohort. Based on the mean change from baseline at 6 months, 116 patients and 68 patients were divided into responders and non-responders, respectively. The mean age of responders at baseline was 64.84 ± 8.08 years, and the mean baseline BCVA was 50.24 ± 14.83 ETDRS letters. The mean age of non-responders at baseline was 66.41 ± 7.95 years, and the mean baseline BCVA was 49.49 ± 15.48 letters. The mean change in BCVA from baseline at 6 months of responders and non-responders was 16.87 ± 8.17 and -1.37 ± 7.32 ETDRS letters, respectively. We found no statistically significant difference in baseline BCVA and age between the responders and non-responders ($p = 0.74$ and 0.20, respectively). The result of Chi-square test indicated no sta-

Table 3. Genotype frequencies of *rs10490924* and *rs11200638* among responders and non-responders at 6 months and 12 months

Time point	dbSNP ID	Genotype	No. (%)		*P* value (2 df)[a]
			Responders	Non-responders	
At 6 months	rs10490924	GG	20 (17)	5 (7)	0.018
		GT	48 (41)	21 (31)	
		TT	48 (41)	42 (62)	
	rs11200638	GG	21 (18)	5 (7)	0.008
		GA	48 (41)	20 (29)	
		AA	47 (41)	43 (63)	
At 12 months	rs10490924	GG	21 (17.5)	4 (6.3)	0.013
		GT	49 (40.8)	20 (31.3)	
		TT	50 (41.7)	40 (62.5)	
	rs11200638	GG	22 (18.3)	4 (6.3)	0.006
		GA	49 (40.8)	19 (29.7)	
		AA	49 (40.8)	41 (64.1)	

Note: SNP, Single nucleotide polymorphism (dbSNP ID); df, degree of freedom. *P* value (2 df): genotype frequencies in responders and non-responders were compared using a Chi square test with 2 df. A *P* value of 0.008 (= 0.05/6) was considered statistically significant after Bonferroni correction.

tistical significance in gender distribution and number of conbercept injections between groups ($p = 0.43$ and 0.37, respectively). Compared with the non-responders, the mean change in BCVA from baseline at 6 months was significantly higher in the responders ($p < 0.05$). The clinical characteristics of the responders and non-responders at 12 months are listed in Table S2 (available at http://www.aaojournal.org).

Association between individual SNP and treatment outcome

The genotype distributions of selected SNPs among responders and non-responders at 6 months and 12 months are presented in Tables S3 and S4 (available at http://www.aaojournal.org), respectively. Genotype frequencies of *rs10490924* and *rs11200638* among responders and non-responders are shown in **Table 3**. The X^2 test revealed the distribution of genotypes for each genetic variant was consistent between responders and non-responders, with the exception of SNPs *rs10490924* and *rs11200638* ($p = 0.018$ and 0.008, respectively) at 6 months. After Bonferroni correction, the significance remained for *rs11200638* ($p < 0.05$) and it became insignificant for *rs10490924* ($p > 0.05$). At 12 months, the distributions of genotype were similar to that at 6 months.

In addition, we compared genotypes for *rs10490924* and *rs11200638* of non-responders at 6 months and 12 months. The genotypes are shown in Tables S5 and S6 (available at http://www.aaojournal.org), respectively. At 6 months, as shown in Table S5, 42 of non-responders had TT genotype, 21 of them had GT genotype, while only 5 of them had GG genotype, for *LOC387715/ARMS2 rs10490924*. As for *rs11200638*, 43 of them had AA genotype, 20 of them had AG genotype, while only 5 of them had GG genotype. At 12 months, genotypes for *rs10490924* and *rs11200638* of non-responders (shown in Table S6) were similar to that at 6 months.

In the subsequent logistic regression analyses, we observed statistically significant associations between treatment outcome and genotypes after adjustment for gender, age and baseline BCVA. As shown in **Table 4**, patients with the GG or GA genotypes at SNP *rs11200638* in the *HTRA1* gene were 2.4 times (OR, 2.43; 95% CI, 1.30-4.52) more likely to be responders after 6 months treatment compared with AA genotypes carriers (uncorrected p = 0.005). For *rs10490924* in the *LOC387715/ARMS2* gene, the presence of the G allele (GG or GT genotypes) was also associated with better outcome to conbercept treatment after 6 months with an uncorrected *p* value of 0.013, but no significance remained after multiple corrections. For other SNPs, no significant association with treatment outcome was observed after 6 months of treatment. At 12 months, the association of the SNP *rs11200638* with treatment outcome remained significant (uncorrected $p = 0.004$), while nominally significant association was found for *rs10490924* (uncorrected $p = 0.009$).

Association between haplotypes and treatment outcome

Linkage disequilibrium analysis was performed by Haploview version 4.2 with use of data from

Table 4. Odds ratios and *p* values for the association of each SNP with the likelihood of a patient being in the responder group versus non-responder group

SNP ID	Genotype	At 6 months		At 12 months	
		OR (95% CI)	*P*-Value*	OR (95% CI)	*P*-Value*
rs1329428	CC	1.0		1.0	
	TT or TC	1.48 (0.80-2.75)	0.209	1.45 (0.78-2.7)	0.241
rs1061170	TT	1.0		1.0	
	CC or CT	0.81 (0.37-1.75)	0.594	0.48 (0.22-1.03)	0.058
rs2227306	CC	1.0		1.0	
	TT or TC	1.29 (0.70-2.36)	0.415	0.95 (0.51-1.75)	0.864
rs10490924	TT	1.0		1.0	
	GG or GT	2.19 (1.18-4.06)	0.013	2.31 (1.23-4.34)	0.009
rs11200638	AA	1.0		1.0	
	GG or GA	2.43 (1.30-4.52)	**0.005**	2.54 (1.35-4.78)	**0.004**
rs2250656	TT	1.0		1.0	
	CC or CT	1.00 (0.54-1.85)	0.992	1.04 (0.56-1.94)	0.904

Note: CI, confidence interval; OR, odds ratio. Ordinary logistic regression modeling adjusted for age, sex and baseline BCVA was used for the analysis. Significance accepted at $p < 0.008$ (bold).

Table 5. Estimated haplotype frequencies among all subjects, responders and non-responders

Haplotypes		Frequency				
		Overall	At 6 months		At 12 months	
			Responders	Non-responders	Responders	Non-responders
G	A	0.003	NA	0.007	NA	0.008
G	G	0.321	0.386	0.221	0.379	0.211
T	A	0.671	0.605	0.772	0.612	0.781
T	G	0.005	0.009	NA	0.009	NA

Note: As listed in **Table 5** based on the marker order: *rs10490924* and *rs11200638*.

all patients. As shown in Figure S1 (available at http://www.aaojournal.org), a small haplotype block composed of *rs10490924* and *rs11200638* was found. The two variants alleles had strong linkage disequilibrium with the r^2 value of 0.96 and the D' value of 0.98. Other variants showed no LD with each other. Since the *rs10490924* and *rs11200638* variants were in linkage disequilibrium in our cohort, this pair of SNPs was combined to process haplotype analysis.

The association between treatment outcome and haplotypes composed of *rs10490924* and *rs11200638* SNPs was evaluated by using the *Haplo.stat* package. **Table 5** shows the frequencies for the estimated haplotypes among all subjects, responders and non-responders at 6 months and 12 months. We observed haplotypes GG and TA based on the marker order: *rs10490924* and *rs11200638* that accounted for more than 99% of all possible marker combinations both in the responder and non-responder samples at 6 months and 12 months. Haplotype analyses identified significant associations between haplotypes and treatment response to conbercept at 6 months (*P* values of 0.01 and 0.007 under the dominant effect) and global simulated *P* values were 0.008 and 0.008 at 12 months. Results from haplotype-specific tests are presented in **Table 6** and indicate significant association between *rs10490924* and *rs11200638* and therapeutic responses under the dominant effect, which were consistent with results of the single-locus analysis.

Discussion

This is the first pharmacogenetic study of treatment for neovascular AMD with conbercept in a Chinese population. In this study, we analyzed 7 SNPs of 6 genes in 184 neovascular AMD patients. We found that, both in single-locus analysis and haplotype analysis, *rs10490924* and *rs11200638* were significantly associated with patient response to conbercept treatment for neovascular AMD in this Chinese population. These findings suggest that SNPs *rs10490924* and *rs11200638* could be used as genetic biomarkers for the estimation of visual outcomes in response to conbercept treatment for neovascular AMD. Furthermore, this will be useful for developing therapeutic strategies for treatment for neovascular AMD with conbercept.

Table 6. Haplotypes most strongly associated with treatment response to conbercept

Haplotypes		At 6 months			At 12 months		
		Score	*P*-value		Score	*P*-value	
			X^2	Simulation		X^2	Simulation
G	G	2.86	0.004	0.004	2. 92	0.003	0.003
T	A	-2.02	0.043	0.026	-2.22	0.026	0.029

Note: As listed in **Table 6** based on the marker order: rs10490924 and rs11200638. Significance accepted at $p < 0.05$.

There have long been controversies in the associations of *ARMS2* and *HTRA1* with anti-VEGF responses [19-21, 27-30]. In congruence with our findings, Tian and coworkers revealed that genotypes of these two genes influenced patients' response to anti-VEGF treatment for neovascular AMD in the Chinese population [23]. They found that mean visual acuity score (VAS) changes were 3.6, 12.1, and 9.6 letters for the TT, TG, and GG genotypes carriers ($p = 0.001$) for *ARMS2 rs10490924*, and for *HTRA1 rs11200638*, mean VAS changes were 3.6, 12.3, and 9.6 letters for the AA, AG and GG genotypes ($p < 0.001$). In a recent Korean neovascular AMD cohort, *rs10490924* and *rs11200638* were also reported to be associated with response of anti-VEGF treatment [31]. However, pharmacogenetic studies from CATT or IVAN trials found no evidence of association [32]. The cause of the inconsistency in the association between genetic variants of these two genes and treatment response may lie in ethnic disparities. In addition, a previous study reported that *ARMS2 rs10490924* as the risk of major AMD variant in Europeans cannot be generalized to a non-European population [33]. Therefore, these two pharmacogenetic associations of anti-VEGF drugs are probably unique among East Asian AMD patients.

The influence of genetic variants of the *CFH Y402H* (*rs1061170*) on the response to anti-VEGF treatment has been the most frequently investigated, but inconsistencies have also been found in previous studies [34]. Barbara and Christian found that patients homozygous for the *CFH Y402H* risk allele (CC genotype) had worse visual outcomes than CT or TT genotypes after anti-VEGF treatment [19, 35]. In contrast, Park and coworkers reported no significant association between the *CFH Y402H* genotype and visual acuity change after anti-VEGF treatment in Korean neovascular AMD patients [31]. A recent meta-analysis revealed that pharmacogenetics of *CFH Y402H* polymorphism may exert an influence on response to anti-VEGF treatment for neovascular AMD, especially for Caucasians [36]. Owing to the lower frequency of the risk allele (C) for *CFH Y402H* in East Asians compared to Caucasians, the correlation of *CFH Y402H* with response to anti-VEGF treatment is hard to evaluate properly. In this Chinese cohort, only one patient (0.5%) had the *rs1061170* CC genotype. This patient was classified into the responder group with visual improvement of 16 letters and 18 letters at months 6 and months 12, respectively.

Concerning the possible biological mechanism of *ARMS2/HTRA1* polymorphisms for their effects on the treatment outcome, *ARMS2* and *HTRA1* both are located on chromosome 10q26, where the locus is considered as the second major risk factor for AMD [37]. It has been suggested that *rs10490924* may lead to the change of *ARMS2* protein function and the increase of susceptibility of retinal photoreceptor cells to aging and oxidative damage [32]. This may be the mechanism by which it would increase the risk of developing AMD. However, how it influences the treatment response is still in dispute [38]. As *rs11200638* is located in the promoter region of *HTRA1*, it is proposed to up-regulate the expression of the gene [39, 40]. There has been a hypothesis that overexpression of *HTRA1* might influence the integrity of Bruch's membrane and promote the development of CNV. This might hint that *rs11200638* in *HTRA1* would play a part in regulating CNV and therefore influence the response to conbercept treatment.

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Disclosure of conflict of interest

None.

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Table S1. Characteristics of candidate genetic markers in this cohort at 12 months

dbSNP ID	Gene	Chr	Minor/Major Allele	MAF	HWE *P*-value	
					Responders	Non-responders
rs1329428	CFH	1	T/C	0.33	0.35	1
rs1061170	CFH	1	C/T	0.10	0.54	0.58
rs2227306	IL8	4	T/C	0.33	0.68	0.59
rs3025039	VEGFA	6	T/C	0.17	1	< 0.05
rs10490924	ARMS2	10	G/T	0.32	0.17	0.47
rs11200638	HTRA1	10	G/A	0.33	0.13	0.45
rs2250656	C3	19	C/T	0.23	0.32	0.72

Note: SNP, Single nucleotide polymorphism (dbSNP ID); Chr, Chromosome; MAF, Minor allele frequency; HWE, Hardy-Weinberg Equilibrium. Significance accepted at $p < 0.05$.

Table S2. Comparisons of clinical characteristics of the responders and non-responders at 12 months

Variables	Responders	Non-responders	*P* value[a]
Number of patients	120	64	-
Gender, male/female	81/39 (68)	42/22 (66)	0.62
Age, mean ± SD	65.11 ± 8.34	66.00 ± 7.50	0.48
Number of injections	7.69 ± 2.62	7.75 ± 2.65	0.89
Visual acuity, mean ± SD			
At baseline	49.33 ± 15.29	51.16 ± 14.6	0.43
At 12 months	67.6 ± 17.23	47.81 ± 14.54	0.00*
Change at 12 months	18.28 ± 9.22	-3.34 ± 7.96	0.00*

Note: [a]Two-sided Chi square test and ANOVA test were used to compare clinical characteristics between the responders and non-responders. SD, standard deviation; BCVA, best corrected visual acuity.*Both the difference in the mean change in BCVA from baseline at 12 months and the difference in the BCVA measured at 12 months between the responders and non-responders are statistically significant ($p < 0.05$).

Table S3. Genotype frequencies of selected SNPs of 6 genes among responders and non-responders at 6 months

Gene	dbSNP ID	Genotype	No. (%)		*p* value (2df)[a]
			Responders	Non-responders	
CFH	rs1329428	TT	14 (12)	5 (7)	0.437
		TC	55 (47)	30 (44)	
		CC	47 (41)	33 (49)	
	rs1061170	CC	1 (1)	0 (0)	0.644
		CT	20 (17)	14 (21)	
		TT	95 (82)	54 (79)	
IL8	rs2227306	TT	12 (10)	8 (12)	0.597
		TC	55 (47)	27 (40)	
		CC	49 (42)	33 (49)	
ARMS2	rs10490924	GG	20 (17)	5 (7)	0.018
		GT	48 (41)	21 (31)	
		TT	48 (41)	42 (62)	
HTRA1	rs11200638	GG	21 (18)	5 (7)	0.008
		GA	48 (42)	20 (29)	
		AA	47 (39)	43 (64)	
C3	rs2250656	CC	7 (6)	4 (6)	0.994
		CT	40 (34)	23 (34)	
		TT	69 (59)	41 (60)	

Note: SNP, Single nucleotide polymorphism (dbSNP ID); df, degree of freedom. [a]*P* values [2 degrees of freedom (df)]: genotype frequencies in responders and non-responders were compared using a Chi square test with 2 df. A *P* value of 0.008 (= 0.05/6) was considered statistically significant after Bonferroni correction.

Table S4. Genotype frequencies of selected SNPs of 6 genes among responders and non-responders at 12 months

Gene	SNP ID	Genotype	No. (%)		*P* value (2 df)[a]
			Responders	Non-responders	
CFH	rs1329428	TT	16 (13.3)	3 (4.7)	0.166
		TC	55 (45.8)	30 (46.9)	
		CC	49 (40.8)	31 (48.4)	
	rs1061170	CC	1 (0.8)	0 (0.0)	0.096
		CT	17 (14.2)	17 (26.6)	
		TT	102 (8.3)	47 (73.4)	
IL8	rs2227306	TT	11 (9.2)	9 (14.1)	0.589
		TC	55 (45.8)	27 (42.2)	
		CC	54 (45.0)	28 (43.8)	
ARMS2	rs10490924	GG	21 (17.5)	4 (6.3)	0.013
		GT	49 (40.8)	20 (31.3)	
		TT	50 (41.7)	40 (62.5)	
HTRA1	rs11200638	GG	22 (18.3)	4 (6.3)	0.006
		GA	49 (40.8)	19 (29.7)	
		AA	49 (40.8)	41 (64.1)	
C3	rs2250656	CC	9 (7.5)	2 (3.1)	0.487
		CT	40 (33.3)	23 (35.9)	
		TT	71 (59.2)	39 (60.9)	

Note: SNP, Single nucleotide polymorphism (dbSNP ID); df, degree of freedom. [a]*P* value (2 df): genotype frequencies in responders and non-responders were compared using a chi square test with 2 df. A *P* value of 0.008 (= 0.05/6) was considered statistically significant after Bonferroni correction.

Table S5. Comparison of genotype for *rs10490924* and *rs11200638* of non-responders at 6 months

Patient Number	rs10490924	rs11200638	BCVA changes
1	GG	GG	1
3	TT	AA	0
5	TT	AA	3
6	GT	AA	3
7	GT	AG	2
8	GT	AG	0
11	GT	AG	1
14	TT	AA	-2
16	TT	AA	-6
18	GT	AG	-11
19	GG	GG	-3
20	TT	AA	2
21	TT	AA	0
22	TT	AA	2
23	TT	AA	-5
26	GT	AG	-3
27	TT	AA	2
28	GT	AG	-20
30	GT	AG	1
33	GT	AG	-1
38	TT	AA	-27
40	TT	AA	3
43	TT	AA	-6
45	GT	AG	-3
47	TT	AA	2
50	GT	AG	-1
55	GT	AG	2
58	TT	AA	2
59	GT	AG	5
68	GT	AG	4
69	GT	AG	-1
71	TT	AA	3
72	TT	AA	0
76	GT	AG	-10
81	GG	GG	5
84	TT	AA	2
88	TT	AA	3
92	TT	AA	-24
94	TT	AA	-2
100	TT	AA	4
102	TT	AA	0
106	TT	AA	-4
107	TT	AA	3
114	GT	AG	1
118	GT	AG	-2
120	TT	AA	4
125	GT	AG	5
126	TT	AA	0
127	TT	AA	3
129	TT	AA	2
134	TT	AA	4
140	TT	AA	-1
142	TT	AA	5
143	GT	AG	-3
147	GT	AG	0
148	TT	AA	-3
149	TT	AA	2
150	TT	AA	2
152	TT	AA	-9
155	TT	AA	-34
159	GG	GG	0
161	TT	AA	5
164	TT	AA	-2
169	TT	AA	4
172	TT	AA	-5
174	TT	AA	5
182	TT	AA	-3
183	GG	GG	1

Note: BCVA, best corrected visual acuity.

Table S6. Comparison of genotype for *rs10490924* and *rs11200638* of non-responders at 12 months

Patient Number	rs10490924	rs11200638	BCVA changes
2	TT	AA	0
5	TT	AA	4
6	GT	AA	3
7	GT	AG	1
8	GT	AG	0
11	GT	AG	1
14	TT	AA	-8
16	TT	AA	-8
18	GT	AG	-15
19	GG	GG	-4
20	TT	AA	-2
21	TT	AA	0
23	TT	AA	-5
27	TT	AA	5
28	GT	AG	-13
30	GT	AG	1
38	TT	AA	-20
43	TT	AA	-6
45	GT	AG	-6
50	GT	AG	3
52	TT	AA	-8
55	GT	AG	4
57	GG	GG	-8
58	TT	AA	-4
69	GT	AG	-2
71	TT	AA	2
72	TT	AA	2
74	GT	AG	-12
83	TT	AA	-9
84	TT	AA	-1
88	TT	AA	2
92	TT	AA	-21
94	TT	AA	5
97	TT	AA	-11
98	TT	AA	5
102	TT	AA	1
106	TT	AA	0
108	TT	AA	-1
114	GT	AG	1
118	GT	AG	4
125	GT	AG	-2
126	TT	AA	5
127	TT	AA	-18
129	TT	AA	5
134	TT	AA	4
136	TT	AA	0
137	GT	AG	-14
140	TT	AA	1
142	TT	AA	3
143	GT	AG	4
147	GT	AG	0
148	TT	AA	1
149	TT	AA	3
152	TT	AA	-22
155	TT	AA	-25
159	GG	GG	2
160	GT	AG	5
161	TT	AA	3
163	GG	GG	1
164	TT	AA	0
167	TT	AA	-16
172	TT	AA	-12
180	GT	AG	-2
182	TT	AA	-20

Note: BCVA, best corrected visual acuity.

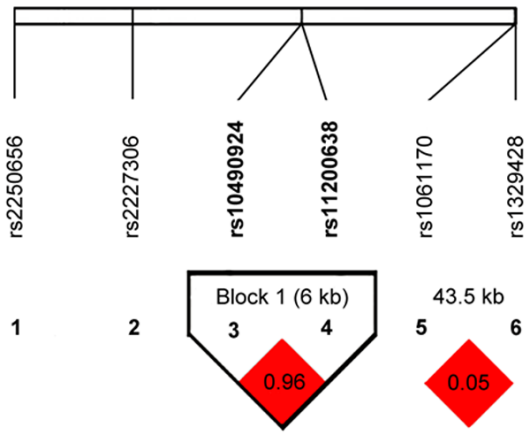

Figure S1. Linkage disequilibrium between 6 SNPs. LD was measured with use of data from all patients. LD calculation (r^2) was shown by the LD map.