Meta-Analysis of the Pharmacogenetics of *ARMS2* A69S Polymorphism and the Response to Advanced Age-Related Macular Degeneration

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Keywords

Age-related macular degeneration · *ARMS2* · Polymorphism · Anti-vascular endothelial growth factor · Meta-analysis

Abstract

Age-related macular degeneration (AMD) causes irreversible vision loss, and targeted anti-vascular endothelial growth factor (VEGF) therapy is now the most common and effective treatment. The aim of this meta-analysis is to discuss whether genetic polymorphism of *ARMS2* A69S could confer susceptibility to advanced AMD with the response to anti-VEGF treatment. We performed a meta-analysis of relevant published studies selected through electronic databases. A total of 21 preferred studies regarding the association between *ARMS2* gene and anti-VEGF treatment response in advanced AMD were generally included in the meta-analysis. The pooled results demonstrated that the carriage of G allele for *ARMS2* A69S presented a better clinical prognosis for advanced AMD treated with anti-VEGF drugs (OR = 1.38, 95% CI = 1.13–1.69, $p = 0.002$). In addition, in the subgroup analysis based on ethnicity, *ARMS2* polymorphisms were more likely to be a positive responder for East Asian patients (OR = 1.67, 95% CI = 1.29–2.16, $p < 0.001$). This meta-analysis

through a series of rigorous methodology data demonstrated a significant association between *ARMS2* A69S polymorphism and the anti-VEGF treatment response in advanced AMD, especially among East Asian population. Numerous well-designed, randomized, multicenter clinical trials with large sample size are required to validate the association.

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Introduction

Age-related macular degeneration (AMD) is a chronic, progressive condition presenting untreatable in up to 90% of patients and is a major cause of irreversible blindness in the elderly individuals [1]. AMD primarily affects people aged older than 50 and implicates a population of 30–50 million worldwide [2]. About two-thirds of people aged older than 80 years are afflicted by AMD to some degree in USA [3], while the prevalence of AMD among Asian population aged 40–79 is estimated at 6.8% [4]. AMD is classified into 2 types, dry and wet, based on the presence or absence of choroidal neovascularization (CNV) and bleeding, which is further differentiated into exudative AMD (advanced AMD) and geographic atro-

phy (late “dry” AMD). In advanced AMD, CNV breaks through to the neural retina, causing the leakage of fluid, lipids, and blood, thus leading to fibrous scarring. Although the etiology of AMD is multifactorial, some common risk factors including aging, smoking, gender, hypertension, obesity, and lifetime light exposure have certain epidemiologic associations with its progression [5].

It has been demonstrated that vascular endothelial growth factor (VEGF) could be extracted in choroidal neovascular membranes obtained from AMD patients [6]. Besides, the VEGF levels in the aqueous humor of AMD patients were significantly increased as compared with control eyes without ocular or systemic diseases [7]. In animal models, the expression of VEGF has been identified in experimental CNV, and it presented tendency to induce the neovascularization [8]. Intravitreal treatment with anti-VEGF drugs, such as ranibizumab, aflibercept, and bevacizumab, remains a valid approach to tackle the progression of advanced AMD [9], which dramatically intensifies or stabilizes visual acuity in a large number of patients. Recently, a long time-span prospective study has corroborated that legal blindness attributable to AMD could be reduced by half in some countries when introducing VEGF antagonists [10].

Current targeted therapies for advanced AMD are based on VEGF inhibition. However, we should take into account the potential risk of long-term consequences of intraocular VEGF suppression on the retina due to inhibition of physiological VEGF function. A large community of AMD patients receiving anti-VEGF treatment could not have their lost vision properly improved or recovered, even with persistent macular edema, despite continuous injection of bevacizumab or ranibizumab [11, 12]. Other etiologies such as genetic backgrounds may illustrate the likelihood. Just as Andreoli et al. [13] pointed out that each individual would have a characteristic genome phenotype toward intravitreal injection, we have had an eye on gene polymorphism as to this tough problem.

Though the most significant loci associated with advanced AMD are focused on the gene for complement factor H located on chromosome 1q31, increasing evidence from the latest large case-control genome-wide association studies confirmed the association with other genes involved as candidate risk factors. The polymorphisms of age-related maculopathy susceptibility 2 (*ARMS2*, also called LOC387715) gene at the 10q26 locus have been reported to be strongly associated with the inverse effect of hormone replacement therapy on AMD. Investigators have demonstrated that the encoded mRNA

and corresponding peptide are expressed in the retina, denoting that *ARMS2* transcript takes the responsibility for the relationship with AMD [14]. Besides, the *ARMS2* protein has been described as a component of the extracellular matrix and its mRNA owns a unique splice form in the retina but commonly no in other tissues [15, 16]. Polymorphic site A69S (rs10490924) within the *ARMS2* locus was mainly investigated in several studies in regard to AMD, and the results showed strong correlation of poor visual acuity in advanced AMD with the response of anti-VEGF intervention [13].

Although several studies have been reasonably substantiated that *ARMS2* A69S polymorphism conferred susceptibility to advanced AMD, others have declared no such associations. We postulated that these disparities are probably triggered by small sample size, ethnic difference, low statistical power, or clinical heterogeneity. In order to better understand the natural association of *ARMS2* genetic polymorphism with the anti-VEGF treatment response in advanced AMD, we conducted a systematic meta-analysis. The present study aimed to determine whether *ARMS2* A69S polymorphism plays a crucial role in regulating the risk of advanced AMD.

Materials and Methods

Search Strategy

A comprehensive online search for relevant studies in the common electronic databases including PubMed, Web of Science, EMBASE, and Cochrane Library was performed by 2 independent researchers for the meta-analysis. Search terms in this study were “gene,” “polymorphism,” “SNP,” “variant,” “macular degeneration,” “age-related macular degeneration,” “AMD,” “age-related maculopathy susceptibility 2,” “*ARMS2*,” “LOC387715,” “anti-vascular endothelial growth factor,” “anti-VEGF,” and “anti-angiogenesis.” Search syntaxes such as “OR” and “AND” were used between those search terms. The studies were searched without any limitation on language, ethnicity, and publication year. In addition, the reference lists from retrieved studies were also checked to identify the source of any relevant ones that were not yet retrieved by our computerized databases. The final search was up-to-date as of November 8, 2019.

Literature Screening

The available studies retrieved from electronic databases were inspected in terms of the title, keywords, abstract, and full text in our meta-analysis. Any disputes were resolved by group consensus through discussion in collaboration with a third researcher. Studies were conceived eligible for inclusion if they met the following criteria: (a) evaluation on the relationship between *ARMS2* A69S polymorphism and the anti-VEGF treatment response in advanced AMD; (b) independent retrospective or prospective study; (c) detailed numbers or percentages in genotype distribution of *ARMS2* A69S could be obtained in patients; (d) the study subjects

Fig. 1. Flow diagram presenting the result of literature searching process in meta-analysis.

were human beings; and (e) polypoidal choroidal vasculopathy (PCV), as a subtype of advanced AMD, was generally considered in our meta-analysis. Results from previous article, conference, case report, review, opinion, letter, and insufficient raw data after contacting with the corresponding author were extracted and excluded.

Data Extraction and Quality Assessment

Detailed data were carefully extracted from our eligible studies independently by 2 researchers (J.Z. and Z.L.). The collected data from each study contained first author, publication year, nationality and racial makeup of the study population, study size, characteristics of included cases, information on study design, frequency or percentage in genotype distribution of ARMS2 A69S, treatment modalities, and study endpoint. Patients who underwent the clinical follow-up ranged from 3 to 24 months and at least 3 initial monthly injections of anti-VEGF therapy were required for inclusion. Clinical outcome measures of the positive response to treatment were based on visual acuity and anatomical features of advanced AMD assessed by optical coherence tomography (OCT), fundus fluorescein angiography, and indocyanine green angiogra-

phy. The quality evaluation of the selected studies was also performed by 2 researchers (J.Z. and Z.L.) using the Newcastle-Ottawa scale [17]. A study with >6 stars was regarded as high quality, and one with 9 stars was regarded as the highest quality. The listed information was rigorously inspected by 2 researchers (J.Z. and Z.L.), and an absolute consensus on all respects of an included paper was reached without any disputes. Any disagreement would be validated and resolved by a third researcher (S.H.).

Statistical Analysis

A series of quantitative analyses were performed by using the statistical software STATA V.12.0 (StataCorp LP, College Station, TX, USA). The odds ratio (OR) and 95% confidence interval (CI) were calculated, and pooled ORs were evaluated for the comparison on the frequency of ARMS2 A69S polymorphism in advanced AMD patients. Cochran's Q test was used to identify potential heterogeneity ($p < 0.1$, treated as significant level across all studies). Moreover, the quantitative I^2 statistic was utilized for evaluation of inconsistency in our meta-analysis, representing the percentage of the observed variability due to heterogeneity rather than chance. The quantification of heterogeneity was assigned of low, moderate,

Table 1. The general characteristics of all studies included in our meta-analysis

References	Year	Country	Ethnicity	Cases, <i>n</i>	Intervention	Mean age, years	Follow-up, months	Endpoint	Quality score
Brantley et al. [18]	2007	America	Caucasian	86	IVB	79.8	6	VA (Snellen)	8
Orlin et al. [19]	2012	America	Caucasian	149	IVR/IVB	80.6	24	VA (Snellen)	7
Yamashiro et al. [21]	2012	Japan	East Asian	77	IVR	75.1	12	OCT	6
Park and Kim [20]	2012	Korea	East Asian	51	IVB + PDT	70.1	12	ICGA	7
Abedi et al. [22]	2013	Australia	Caucasian	206	IVR/IVB	78	12	VA (ETDRS)	7
Lotery et al. [27]	2013	UK	Caucasian	254	VEGF inhibition	77.7	12	TRT	6
Kitchens et al. [26]	2013	America	Caucasian	100	IVR/IVB	80	4	OCT	7
Hautamaki et al. [25]	2013	Finland	Caucasian	96	IVB	78	3.5	OCT	6
Hagstrom et al. [24]	2013	America	Caucasian	834	IVR/IVB	78.5	12	VA (ETDRS)	7
Fang et al. [23]	2013	China	East Asian	143	IVB	68.8	6	VA (ETDRS)	6
Park et al. [29]	2014	Korea	East Asian	273	IVR	69.5	5	VA (ETDRS)	7
Matsumiya et al. [28]	2014	Japan	East Asian	120	IVR	76	3	OCT	8
van Asten et al. [30]	2014	Netherland	Caucasian	391	IVR	80	3	VA (ETDRS)	6
Kuroda et al. [32]	2015	Japan	East Asian	236	IVR	74.3	12	OCT	8
Hata et al. [31]	2015	Japan	East Asian	77	IVR	75.8	24	VA (ETDRS)	7
Chaudhary et al. [34]	2016	Canada	Caucasian	70	IVR	80.8	6	VA (ETDRS)	7
Bardak et al. [33]	2016	Turkey	Middle east	39	IVR	NA	3	OCT	8
Kikushima et al. [35]	2017	Japan	East Asian	140	IVA	75	12	OCT	6
Yamashiro et al. [37]	2017	Japan	East Asian	426	IVA	73.6	12	OCT	8
Park et al. [36]	2017	Korea	East Asian	95	IVA	67.6	12	VA (ETDRS)	7
Mohamad et al. [38]	2019	Malaysia	East Asian	145	IVA	69.1	6	VA (Snellen)	8

VEGF, vascular endothelial growth factor; OCT, optical coherence tomography; VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; ICGA, indocyanine green angiography; TRT, total retinal thickness.

Table 2. The main results of all studies included in our meta-analysis

References	Year	Design	Definition of a positive response	Cases with positive response, <i>n</i>					Cases, <i>n</i>				
				G	GG	GT	TT	T	G	GG	GT	TT	T
Brantley et al. [18]	2007	Retrospective	Improved visual acuity	47	15	17	6	29	104	33	38	15	68
Orlin et al. [19]	2012	Retrospective	Improved/unchanged visual acuity	107	35	37	21	79	173	55	63	31	125
Yamashiro et al. [21]	2012	Retrospective	Retinal exudate resolved by OCT	38	9	20	18	56	61	16	29	32	93
Park et al. [20]	2012	Retrospective	Complete polyp regression	39	12	15	11	37	42	12	18	21	60
Abedi et al. [22]	2013	Prospective	<15 letters of vision loss	NA	146		33	NA	NA	162		44	NA
Lotery et al. [27]	2013	Prospective	≥75th percentile in TRT change	126	NA	NA	NA	126	280	NA	NA	NA	228
Kitchens et al. [26]	2013	Retrospective	No retinal fluid at the fourth month	110	38	34	7	48	129	45	39	16	71
Hautamaki et al. [25]	2013	Prospective	Retinal exudate resolved by OCT	47	10	27	7	41	98	21	56	19	94
Hagstrom et al. [24]	2013	Prospective	Gain of 15 or more letters	280	77	126	48	222	930	266	398	170	738
Fang et al. [23]	2013	Prospective	Gain of 15 or more letters	33	6	21	19	59	76	16	44	83	210
Park et al. [29]	2014	Prospective	Gain of 8 or more letters	96	19	58	59	176	181	36	109	128	365
Matsumiya et al. [28]	2014	Retrospective	No retinal fluid or edema	84	23	38	35	108	103	27	49	44	137
Van Asten et al. [30]	2014	Prospective	<30% letters of visual acuity loss	374	115	144	85	314	420	127	166	98	362
Kuroda et al. [32]	2015	Retrospective	No retinal hemorrhage or fluid	47	11	25	27	79	132	33	66	76	218
Hata et al. [31]	2015	Prospective	Improved/unchanged visual acuity	39	10	19	25	69	52	10	32	35	102
Chaudhary et al. [34]	2016	Prospective	Gain of 15 or more letters	30	11	8	4	16	84	27	30	13	56
Bardak et al. [33]	2016	Prospective	Absence of retinal fluid	9	3	3	5	13	34	10	14	15	44
Kikushima et al. [35]	2017	Retrospective	Retinal fluid resolved by OCT	50	15	20	11	42	110	26	58	56	170
Yamashiro et al. [37]	2017	Prospective	Absence of intra- or subretinal fluid	136	42	52	36	124	361	90	181	155	491
Park et al. [36]	2017	Prospective	<5 letters of visual acuity loss	21	4	13	8	29	57	11	35	34	103
Mohamad et al. [38]	2019	Prospective	Improvement of 3 lines or greater	54	16	22	16	54	99	21	57	67	191

OCT, optical coherence tomography.

Table 3. The main results of pooled ORs and analysis of *ARMS2* gene polymorphism with advanced AMD in our meta-analysis

Polymorphism (rs10490924)	Ethnicity	Studies, <i>n</i>	Test of heterogeneity			Test of association		
			I^2 , %	P_H	model	OR	95% CI	<i>p</i> value for test
G versus T (allelic)	Overall	20	69.1	<0.001	R	1.38	(1.13, 1.69)	0.002
	Caucasian	8	55.5	0.028	R	1.10	(0.86, 1.40)	0.455
	East Asian	11	61.1	0.004	R	1.67	(1.29, 2.16)	<0.001
	Middle East	1	NA	NA	NA	0.86	(0.32, 2.33)	0.765
GG versus TT (homozygote)	Overall	19	53.6	0.003	R	1.88	(1.32, 2.67)	<0.001
	Caucasian	7	31.9	0.184	F	1.25	(0.92, 1.70)	0.147
	East Asian	11	55.1	0.014	R	2.38	(1.44, 3.93)	0.001
	Middle East	1	NA	NA	NA	0.86	(0.15, 4.82)	0.861
GT versus TT (heterozygote)	Overall	19	29.2	0.114	F	1.36	(1.14, 1.62)	0.001
	Caucasian	7	42.8	0.106	F	1.20	(0.91, 1.60)	0.201
	East Asian	11	19.2	0.260	F	1.50	(1.19, 1.88)	<0.001
	Middle East	1	NA	NA	NA	0.55	(0.10, 2.89)	0.476
GG + GT versus TT (dominant)	Overall	20	46.7	0.012	R	1.62	(1.27, 2.06)	<0.001
	Caucasian	8	54.5	0.031	R	1.52	(0.98, 2.35)	0.063
	East Asian	11	40.6	0.078	R	1.76	(1.31, 2.36)	<0.001
	Middle East	1	NA	NA	NA	0.67	(0.16, 2.75)	0.575
GG versus GT + TT (recessive)	Overall	19	47.3	0.012	R	1.51	(1.15, 2.00)	0.004
	Caucasian	7	0.0	0.718	F	1.09	(0.87, 1.38)	0.460
	East Asian	11	53.3	0.018	R	1.90	(1.21, 2.98)	0.005
	Middle East	1	NA	NA	NA	1.13	(0.23, 5.45)	0.884

AMD, age-related macular degeneration; OR, odds ratio; CI, confidence interval; NA, not available.

high, and extremely high to I^2 values of 0–25, 25–50, 50–75, and 75–100%, respectively. Either the fixed-effect model ($I^2 < 50\%$ and $p > 0.1$) or random-effect model ($I^2 \geq 50\%$ and $p < 0.1$) was applied for the pooled ORs and 95% CIs according to the heterogeneity. We also performed sensitivity analysis to assess the effect of each study on the pooled ORs by omitting one study in each turn. Moreover, subgroup analysis was executed to apprehend the strength of association between different ethnicities. A series of meta-regression analyses were applied for the discussion of main heterogeneity. Publication bias was estimated by Begg's test with the funnel plot and Egger's regression test. p value <0.05 was considered statistically significant in these comparisons.

Results

General Characteristics of Selected Studies and Quality Assessment

A flow diagram of the study selection process is presented in Figure 1. The search strategy resulted in 223 relevant articles from the 4 databases. Of which, 175 studies were excluded for duplication (156 articles) and ineligible categories such as abstract, review, conference, and case

report (19 articles). The rest 48 full-text articles were left in our meta-analysis. Among them, 11 studies did not provide detailed genotype results after perusing the documents and additional files and 16 articles did not discuss the association between *ARMS2* and anti-VEGF intervention. Finally, a total of 21 preferred studies regarding the association between *ARMS2* gene and the anti-VEGF treatment response in advanced AMD were generally included in the current meta-analysis [18–38]. The main characteristics and data of the enrolled studies are listed in Table 1. Regarding ethnicity, 9 of these studies were performed in Caucasian, 11 in East Asian, and 1 in Middle East people. Four studies used aflibercept, 3 used bevacizumab, and 8 used ranibizumab, while 4 studies used either ranibizumab or bevacizumab, one used bevacizumab or photodynamic therapy, and another one used VEGF inhibition. The follow-up duration ranged from 3 months to 2 years. We classified the definition of positive or negative response based on the improvement on visual acuity or macular morphological changes. Moreover, the quality assessment of all included studies is presented in the Table 1 and all studies were of high quality with >6 stars.

Fig. 2. Evaluation of the association between ARMS2 gene polymorphism (G vs. T) with advanced AMD. AMD, age-related macular degeneration.

Bias Assessment of the Included Studies

The evaluation of potential bias was primarily expounded in Table 2. Thirteen studies were prospective, and 8 were retrospective. Overall, the quality of the included studies was consistently robust. Of the studies, there were no obvious biases in the selection of advanced AMD patients, confounding, or selective outcome reports. The result with definition of a positive responder could be validated in our meta-analysis.

Association between ARMS2 A69S Polymorphism and Advanced AMD Susceptibility with Anti-VEGF Treatment

We meta-analyzed the 21 included studies for the pooled associations between treatment response in advanced AMD and ARMS2 A69S genotypes in Table 3. We summarized the statistical differences involving 5 genotype comparisons (19 studies for G vs. T, GG vs. TT, and GT vs. TT; 20 studies for GG + GT vs. TT and GG vs. GT + TT) of the study population (allelic model: OR = 1.38, 95% CI = 1.13–1.69, $p = 0.002$, random model; homozygote model: OR = 1.88, 95% CI = 1.32–2.67, $p < 0.001$, random model; heterozygote model: OR = 1.36, 95% CI = 1.14–1.62, $p = 0.001$, fixed model; dominant model: OR = 1.62, 95% CI = 1.27–2.06, $p < 0.001$, random model; re-

cessive model: OR = 1.51, 95% CI = 1.15–2.00, $p = 0.004$, random model) (Fig. 2–6). Besides, subgroup analysis based on 3 different racial groups revealed that there were no significant differences between any Caucasian or Middle East genotypic comparison models, but a significant association was indicated in the pooled analyses between A69S and anti-VEGF effects in all genotypic comparisons for East Asian participants (allelic model: OR = 1.67, 95% CI = 1.29–2.16, $p < 0.001$, random model; homozygote model: OR = 2.38, 95% CI = 1.44–3.93, $p = 0.001$, random model; heterozygote model: OR = 1.50, 95% CI = 1.19–1.62, $p = 0.001$, fixed model; dominant model: OR = 1.62, 95% CI = 1.27–2.06, $p < 0.001$, random model; recessive model: OR = 1.51, 95% CI = 1.15–2.00, $p = 0.004$, random model) (Table 3).

Heterogeneity Test and Sensitivity Analysis

Significant heterogeneity between the A69S polymorphism in the ARMS2 gene and the anti-VEGF treatment response in advanced AMD was observed among 4 genotype comparisons (all $p < 0.1$) (Fig. 2, 3, 5, 6). Our subgroup analysis in the homozygote and the recessive model suggested the primary heterogeneity owed to racial origin (Table 3). The pooled ORs for sensitivity analysis remained substantial when omitting 1 study at each time.

Fig. 3. Assessment of the association between ARMS2 gene polymorphism (GG vs. TT) with advanced AMD. AMD, age-related macular degeneration.

Fig. 4. Estimation of the association between ARMS2 gene polymorphism (GT vs. TT) with advanced AMD. AMD, age-related macular degeneration.

Fig. 5. Evaluation of the association between *ARMS2* gene polymorphism (GG + GT vs. TT) with advanced AMD. AMD, age-related macular degeneration.

Fig. 6. Assessment of the association between *ARMS2* gene polymorphism (GG vs. GT + TT) with advanced AMD. AMD, age-related macular degeneration.

Fig. 7. Assessment of the sensitivity analysis between *ARMS2* gene polymorphism (G vs. T) with advanced AMD. AMD, age-related macular degeneration.

Table 4. Meta-regression for the heterogeneity of G versus T comparison in our meta-analysis

Covariate factors	Exp(b)	Std. Err	<i>p</i> value	95% CI
Design	1.33	0.25	0.052	0.99–1.78
Ethnicity	2.52	0.75	0.006	1.35–4.71
Follow-up, months	1.74	0.60	0.130	0.84–3.60
Intervention	1.02	0.33	0.944	0.52–2.01
Mean age, years	4.14	5.75	0.319	0.22–76.4
Cases, <i>n</i>	2.34	0.71	0.011	1.24–4.41

Our results demonstrated that no study absolutely changed the relationship between *ARMS2* A69S and treatment response in advanced AMD, signifying that the pooled ORs of the present meta-analysis were relatively robust (Fig. 7, others in online suppl. Fig. 1–4; see www.karger.com/doi/10.1159/000508738 for all online suppl. material).

Meta-Regression

We tried to apply meta-regression for analyzing the heterogeneity of G versus T genotype to the greatest extent. The study design, ethnicity, follow-up duration, intervention, mean age, and study size were thoroughly treated as independent covariate factors. As delineated in Table 4, our meta-regression analysis showed no significant correlations between the aforementioned variables

except ethnicity and number of cases (for ethnicity: $p = 0.006$, 95% CI = 1.35–4.71; for number of cases: $p = 0.011$, 95% CI = 1.24–4.41).

Publication Bias

Begg's test with the funnel plot and Egger's regression test were performed to assess the source of publication bias between *ARMS2* A69S and the treatment response in advanced AMD. There were no conspicuous asymmetries of funnel plots in any genotypic comparisons (Fig. 8, others in online suppl. Fig. 5–8); moreover, no statistically significant relationship was found in Egger's regression test, suggesting no apparent evidence of publication bias ($p > 0.05$, Table 5).

Discussion

AMD is the predominant cause for irreversible blindness in senile population worldwide, especially the advanced type. Anti-VEGF treatment is the primary therapy for patients with vision loss caused by advanced AMD [39, 40]. A total of 13 prospective and 8 retrospective studies involving 4,008 cases with the association between anti-VEGF and A69S were included in our current meta-analysis. Our results revealed that the *ARMS2* A69S allele might be a better prognostic factor for advanced AMD. Specifically, the pooled results based on ethnicity

Fig. 8. Estimation of the publication bias between *ARMS2* gene polymorphism (G vs. T) with advanced AMD. AMD, age-related macular degeneration.

indicated that there was a significant association between *ARMS2* A69S and anti-VEGF treatment response in exudative AMD in the East Asian group, but not in the Caucasian or Middle East group. Thus, these findings may enable clinicians to make the best of these effective medications, at least for East Asian patients.

Anti-VEGF treatment has been extended to treat eyes with PCV, which is a more common subtype of exudative AMD in Asians compared with the Caucasians. In our meta-analysis, a group of patients investigated by Park and Kim [20] had considered PCV subtype and received combined treatment consisting of photodynamic therapy with intravitreal bevacizumab and another group of patients in the same condition explored by Hata et al. [31] was treated with ranibizumab. Besides, Park et al. [36] only centered pharmacogenetics on the association of *ARMS2* A69S polymorphism with ranibizumab treatment in PCV. The lack of thoroughly exudative AMD clinical subtypes is one of the major limitations as performing analysis based on the CNV subtypes (PCV and neovascular AMD) could provide more specific information on which exudative AMD is related with the different response to anti-VEGF therapy. The remaining anti-VEGF pharmacogenetic studies of exudative AMD did not precisely delineate whether PCV was considered in the total of collected genotypic data. Unfortunately, we could not rule out the accurate proportion of PCV in the overall exudative AMD patients, though considering that A69S showed significant association only in the East

Table 5. Bias between *ARMS2* genetic polymorphism with advanced AMD in our meta-analysis

Polymorphism (rs10490924)	Publication, <i>n</i>	Publication bias (<i>p</i> value)	
		Begg's test	Egger's test
G versus T	20	0.206	0.092
GG versus TT	19	0.142	0.125
GT versus TT	19	0.889	0.455
GG + GT versus TT	20	0.456	0.240
GG versus GT + TT	19	0.142	0.075

AMD, age-related macular degeneration.

Asian population in this meta-analysis. Furthermore, our results may be partly explained by the use of different anti-VEGF drugs during treatment, inconsistent follow-up endpoint, discrepant dosing intervals, and adjusting for different environmental factors across studies. However, being consistent with the positive summarized effect, removing the genotype of the cases with PCV did not exert any influence on the overall comparison (allelic model: OR = 1.33, 95% CI = 1.08–1.63; homozygote model: OR = 1.77, 95% CI = 1.24–2.55; heterozygote model: OR = 1.36, 95% CI = 1.13–1.63; dominant model: OR = 1.58, 95% CI = 1.24–2.02; recessive model: OR = 1.46, 95% CI = 1.10–1.94) due to its small sample size ($n = 51$, $n = 77$, and $n = 95$, respectively).

Obviously, considerable genetic heterogeneity existed in our current study, though the results of sensitivity analysis by removing 1 study in each turn showed that the present meta-analysis was relatively robust. Our subgroup analysis based on involved races in the study population suggested that heterogeneity is particularly pronounced among East Asian and Middle East population. Meta-regression was carried out to explore the heterogeneity of G versus T allele, and the results indicated that ethnicity and number of cases were the origin of heterogeneous factors. In addition, heterogeneity may also have arisen from the genetic background, sex difference, lifestyle, level of socioeconomic development, genotyping method, and definition of response.

It has been verified that *ARMS2* lies in the AMD susceptibility locus identified on chromosome 10q26 and is commonly expressed in the retina. Evidence has suggested that *ARMS2* variants could bring about dysfunction of the retinal pigment epithelium due to mitochondrial DNA damage that accumulates in the retina and its pigment epithelium. Sobrin et al. [41] suggested that genetic

mutation at this locus has conferred a differential risk for CNV versus geographic atrophy. With respect to the possible biological mechanism, the *ARMS2* gene product has been localized to the mitochondrial outer membrane; it has been proposed that the A69S polymorphism could alter *ARMS2* function and increase the susceptibility of photoreceptor cells to oxidative damage and aging [14]. In addition, the cellular mitochondria are known to be a major source of superoxide anion, implying that damage to the mitochondria would bring about oxidative stress in neovascular AMD possibly affecting the response to anti-VEGF inhibitors. Besides, Kortvely et al. [15] reported that the *ARMS2* protein is mostly confined to choroid pillars in human eyes, corresponding to the principal sites of drusen deposition. Accordingly, abnormal expression of *ARMS2* would exhibit higher susceptibility to the development of drusen and AMD. As such, it is understandable to increase the risk of developing AMD, but the role of nonsynonymous A69S single-nucleotide polymorphism in the pathogenesis of influencing response to anti-VEGF treatment remains unknown. Abedi et al. [22] implied that the loss of structural integrity of Bruch's membrane, driven by oxidative pathways mediated by *ARMS2* A69S variants, may largely contribute to the underlying biological mechanisms for a weak response to intravitreal anti-VEGF treatment.

Yamashiro et al. [37] argued that *ARMS2* A69S was not significantly associated with the changes in visual acuity or anatomic outcomes during the 1-year anti-VEGF treatment in treatment-naïve advanced AMD patients, while Kitchens et al. [26] confirmed that A69S was positively correlated with the achievement of dry maculae after 3 monthly injections of ranibizumab or bevacizumab, suggesting better response of *ARMS2* A69S with advanced AMD. Moreover, Teper et al. [42] indicated a significant influence of the risk homozygosis (TT) for *ARMS2* A69S on the visual response after ranibizumab treatment. The differences could be owed to the small sample size and relatively short-term follow-up period in those studies. We have a large enough sample size containing 4,008 cases in the current meta-analysis; the results strongly demonstrated that G allele carriage or GG homozygosis might be useful for better predicting visual outcomes after anti-VEGF treatment.

Apart from *ARMS2* A69S polymorphism, several variants within the chromosome 10q26 region have been investigated and their association with advanced AMD susceptibility has been demonstrated, such as a *HTRA1* promoter variant rs11200638 adjacent to *ARMS2* and an insertion/deletion variant in the 3' untranslated region of

ARMS2 gene [43]. Moreover, the functional rs11200638 in the promoter region of the *HTRA1* gene exhibited a complete linkage disequilibrium ($D' > 0.99$) with *ARMS2* A69S [44]. However, and by contrast with our results, evidence from a meta-analysis by Zhou et al. [45] showed no influence of pharmacogenetic response with *HTRA1* rs11200638. Some of this inconsistency may be accounted for by the following reasons that the number of eligible studies and included patients by Zhou et al. [45] was less than that in the current study (5 studies vs. 21 studies; 1,570 cases vs. 4,008 cases). In addition, other factors such as epigenetic or environmental conditions also appeared to play a role in the discrepancy. More studies and further rigorous investigations are needed to address this issue.

A previous meta-analysis conducted by Hu et al. [46] has revealed a significant association between *ARMS2* A69S and the response to anti-angiogenesis treatment in advanced AMD patients among 3 genotypic models (homozygote model: OR = 1.34, 95% CI = 1.01–1.77, $p = 0.039$; heterozygote model: OR = 1.58, 95% CI = 1.08–2.31, $p = 0.018$; dominant model: OR = 1.74, 95% CI = 1.19–2.52, $p = 0.004$). Consistently, our meta-analysis also found the significance of the difference in the comparison of G versus T (OR = 1.38, 95% CI = 1.13–1.69, $p = 0.002$). Moreover, our meta-analysis focused on 3 ethnicities including East Asian, Caucasian, and Middle East population, though no significant association was validated in last 2 races. Furthermore, the pooled results in the current study did not delineate any inconsistency in the exploration of sensitivity test and publication bias, which differs from the conclusions from Hu et al. [46]. The explanation for this difference could be drawn on the increasing number of studies and larger sample size, thus guaranteeing our cumulative ORs higher definiteness, stability, and accuracy than before.

In order to accurately interpret our combined results, several potential limitations of the current study should not be ignored. First, heterogeneity based on meta-regression was found in covariate factors including ethnicity and number of cases. Second, the variable criteria in grouping the positive and negative response to the anti-VEGF treatment could also be one of the factors affecting the pharmacogenetic results, as there are contradictory findings in previous studies, though the included ones were given adequate definition of positive and negative responses. Third, the number of patients was relatively small in each study; thus, a research with larger sample size involving different ethnic regions is necessary for further analysis. Fourth, the difference between central

macular thickness and maximum lesion thickness with baseline level was also a meaningful clinical outcome in responsive to anti-VEGF treatment. Unfortunately, our study could not point out any relationship between these 2 clinical outcomes and the polymorphisms. Fifth limitation is that only 3 ethnic backgrounds with relatively small studies were included in this meta-analysis; thus, further efforts to lower the incidence of ethnic bias will be taken into account. Finally, included studies were of high quality, but presenting a challenge that the nonrandomized study design was not considered in this meta-analysis.

Conclusion

The current meta-analysis demonstrated a series of rigorous methodology data for a significant association between *ARMS2* A69S and the anti-VEGF treatment response in advanced AMD, especially in East Asian population. More studies, well-designed work with randomized, multicenter clinical trials or intervention studies incorporating different ethnicities together with gene-gene or gene-environment interaction, are needed to further prove the biofunctional role of this common *ARMS2* polymorphism.

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Statement of Ethics

All analyses were based on previous published studies; thus, no ethical approval and patient consent are required.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest to this work.

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Author Contributions

J.Q. conceived the study. J.Z. and Z.L. performed and checked the available information from eligible articles in this meta-analysis. S.H. analyzed the data. Z.L. prepared Figures 1–8, online suppl. Figures 1–8, and Tables 1–5. J.Z. wrote the main manuscript text. J.Q. reviewed and revised the manuscript. All authors read and approved the final manuscript.

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