LOC387715/HTRA1 gene polymorphisms and susceptibility to age-related macular degeneration: A HuGE review and meta-analysis

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Purpose: To examine the association of age-related macular degeneration (AMD) with HtrA serine peptidase 1 (*HTRA1*) gene rs11200638 G→A polymorphism and *LOC387715/ ARMS2* gene rs10490924 G→T polymorphisms, and to evaluate the magnitude of the gene effect and the possible genetic mode of action.
Methods: We searched the US National Library of Medicine's PubMed, Embase, OMIM, ISI Web of Science, and CNKI databases in a systematic manner to retrieve all genetic association studies on the *HTRA1* (rs11200638) and *LOC387715/ ARMS2* (rs10490924) gene polymorphisms and AMD. We performed a meta-analysis conducted with Stata software, version 9.0.
Results: Individuals who carried the AA and AG genotypes of *HTRA1* gene rs11200638 G→A polymorphism had 2.243 and 8.669 times the risk of developing AMD, respectively, when compared with those who carry the GG genotype. Individuals carrying the TT and TG genotypes of *LOC387715/ ARMS2* gene rs10490924 G→T polymorphism had 7.512 and 2.353 times the risk of developing AMD, respectively, compared with those who carry GG genotype. These results suggested a "moderate" codominant, multiplicative genetic mode; that is, both *HTRA1* rs11200638 G→A polymorphism and *LOC387715/ARMS2* rs10490924 G→T polymorphism play important roles in the pathogenesis of AMD. We found no evidence of publication bias. Between-study heterogeneity was found in both allele-based analysis and genotype-based analysis.
Conclusions: *HTRA1* rs11200638 G→A polymorphism and *LOC387715/ARMS2* rs10490924 G→T polymorphism play important roles in AMD. Gene-gene and gene-environmental interactions, as well as precise mechanisms underlying common variants in the *HTRA1* gene and *LOC387715/ ARMS2* gene, potentially increase the risk of AMD and need further exploration.

Age-related macular degeneration (AMD) is a neurodegenerative disease that leads to visual impairment and accounts for half of all cases of registered blindness in Western individuals older than 65 years of age [1-14]. There are approximately eight million people in the United States with symptoms of early or intermediate AMD, of whom approximately one million will develop advanced AMD within the next five years [15-17]. AMD is estimated to affect about 50 million people worldwide [18-20], and an increase in aging populations makes AMD a significant public health concern and a major focus of research efforts (National Advisory Council).

AMD is a clinically heterogeneous and genetically complex disease, with multiple environmental and genetic risk factors involved [20-25]. While epidemiological studies have linked cigarette smoking, alcohol consumption, light exposure, diet, drugs, and high blood pressure to the risk of AMD [19,23,26-36], familial aggregation and twin studies [37-43] have suggested that genetic variation may also play an important role in the disease. Although AMD has been reported to be associated with genetic variations in the genes of adenosine-triphosphate (ATP)-binding transporter protein 4 [44-46], apolipoprotein E [47-52], excision-repair cross-complementing group 6 [53], fibulin 5 [54], fibulin 6 [55, 56], elongation of very-long-chain fatty acids-like 4 [57-59], factor B/complement component 2 [60], toll-like receptor 4 [61-63], and vascular endothelial growth factor [64], recent genome-wide linkage studies found that genomic regions at chromosomes 1q31–32 and 10q26 may have a bigger role in susceptibility to AMD [65]. The identification of overlapping

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loci on chromosome 1q by several study groups [66-68] indicates that this locus probably harbors a major AMD-associated gene. Recently, the component factor H (*CFH*) gene on chromosome 1q31 has been revealed as the first major AMD-susceptibility gene, perhaps accounting for about 30%–50% of AMD patients. The *CFH Y402H* variant in the *CFH* gene has also been identified as a causal polymorphism in studies of populations other than those of European and North American origin [30,69-78], and a follow-up meta-analysis [79] has confirmed this association in Western populations. Studies in Japan, however, did not show any associations between *CFH Y402H* polymorphism and AMD [80,81], suggesting that there must be some other loci susceptible for AMD. Several studies have showed that a locus at chromosome 10q26 [82-84] of *CFH* may independently contribute to AMD susceptibility [65,76,82-84]. Three genes identified at chromosome 10q26 and associated with the risk of AMD are Pleckstrin Homology Domain-containing Protein Family A member 1, age-related maculopathy susceptibility 2 (*LOC387715/age-related maculopathy susceptibility 2 [ARMS2]*), and high-temperature requirement factor A1 (*HTRA1/PRSS11*) [65,76,82-84]. Thus, AMD appears to be a product of the interaction between multiple loci of susceptibility rather than a collection of single-gene disorders. However, the number of loci involved, the degree of attributable risk conferred, and the interactions between various loci remain obscure.

The *HTRA1* gene spans a 53,366-base region on chromosome 10q26 (124211047–124264413, Gene ID: 5654); it encodes a member of a family of serine proteases expressed in both mouse and human retinas [85,86], and its expression in human fibroblasts increases with aging [87]. HTRA1 appears to regulate the degradation of extracellular matrix proteoglycans. This activity has been considered to facilitate access of other degradative matrix enzymes, such as collagenases and matrix metalloproteinases, to their substrates [88]. Overexpression of HTRA1 alters the integrity of Bruch's membrane, favoring the invasion of choroid capillaries across the extracellular matrix, as occurs in wet AMD. HTRA1 also binds and inhibits transforming growth factor-β (TGF-β), an important regulator of extracellular matrix deposition and angiogenesis [89]. During the years 2006 to 2008, several studies were conducted to investigate the association between HTRA1 gene polymorphisms and AMD. A single-nucleotide polymorphism (rs11200638) in the promoter region of the HTRA1 gene was found to be significantly associated with susceptibility to AMD in studies of Caucasian populations in the US [90-97], Central Europe [98], France [99], and the UK [100]; of East Asian populations in China [101-104] and Japan [105-107]; and of Indian populations in India [108]. Another putative AMD-susceptibility gene, *LOC387715/ARMS2*, has recently been identified. *LOC387715/ARMS2* encodes a deduced 107–amino acid protein with nine predicted phosphorylation sites

and a molecular mass of 12 kDa. Real-time (RT)-PCR analysis demonstrated that *LOC387715/ARMS2* transcripts were expressed in the retina and in a variety of other tissues and cell lines. Transfection experiments in mammalian cells localized the protein to the mitochondrial outer membrane [95]. Up to now, the biologic characterization of this gene has been limited. However, Rivera et al. [109] concluded that the A69S single-nucleotide polymorphism (rs10490924) in exon 1 of the *LOC387715/ARMS2* gene was the most likely susceptibility allele of AMD. Since an individual study may not have sufficient statistical robustness to confirm the association between *HTRA1* and *LOC387715/ARMS2* gene polymorphisms and AMD, we considered that a meta-analysis that combined data from all published studies would provide a more accurate estimate of the extent of association, leading to less risk of false-positive results [110]. Thus, we systematically pooled the results of all available population-based association studies of the *HTRA1* rs11200638 G→A polymorphism, the *LOC387715/ARMS2* rs10490924 G→T polymorphism, and AMD. We attempted to estimate the strength of the genetic association with AMD, as well as the genetic mode of action, and to gauge the extent of heterogeneity in the strength of the associations among different studies.

METHODS

Search strategy and inclusion criteria: We searched the US National Library of Medicine's PubMed, Embase, OMIM, ISI Web of Science, and Chinese National Knowledge Infrastructure (CNKI) databases in a systematic manner to retrieve all genetic association studies on the *HTRA1* (rs11200638) and *LOC387715/ARMS2* (rs10490924) polymorphisms and AMD published before April 2008. The search strategy was based on a combination of the terms (HtrA serine peptidase 1 or *HTRA1*), (*age-related maculopathy susceptibility 2 or LOC387715*), and (age-related macular degeneration or AMD). The references of all computer-identified publications were searched for additional studies, and the PubMed option "Related Articles" was also used to search for potentially relevant papers. Searches were performed by two independent reviewers (B.Z. and J.Y.). We included all published articles regardless the language of publication.

Studies were included if they met the following criteria: 1) The study reported original data from case-control or cohort studies. 2) The alleles and genotypes for the *HTRA1* polymorphism (rs11200638), respectively, were A and G and AA, AG, and GG. 3) The alleles and genotypes for the *LOC387715/ARMS2* polymorphism (rs10490924), respectively, were G and T and GG, GT, and TT. 4) The numbers of subjects possessing each allele and genotype in the AMD and control groups were available. 5) In the case of multiple publications from the same study group, the most complete and recent results were used. We set no restriction

on the source of controls (general population, clinic, or hospital). For those studies where AMD was graded (e.g., drusen, pigment abnormalities in retinal pigment epithelium [RPE], geographic atrophy, and choroidal neovascularization [CNV]), the gradings were combined into a single AMD group.

Data extraction: Data were extracted independently by two investigators (B.Z. and J.Y.), who used recommended guidelines to report on meta-analyses of observational studies [111]. The following data were extracted from the eligible studies: authors, journal title and year of publication, country of origin, selection and characteristics of cases and controls, demographic data, ethnicity of the study population (e.g., Caucasian or East Asian), numbers of eligible and genotyped cases and controls, and genotype distributions in cases, controls, and available subgroups. Furthermore, we examined whether matching had been used; whether there was specific mention of blinding of the genotyping personnel to the clinical status of subjects; whether the genotyping method used had been validated; and whether genotype frequencies in control groups conformed to the Hardy–Weinberg equilibrium (HWE). Any disagreement was adjudicated by a third author (R.L.).

Statistical analysis: We used the odds ratio as the metric of choice and this was estimated for each study. To explore the possible association between *HTRA1* and *LOC387715/ARMS2* polymorphisms and AMD, and to avoid excessive comparisons, we calculated the odds ratio by two methods: allele comparison (the A allele versus the G allele in the *HTRA1* rs11200638 G→A polymorphism), and comparing the risk-variant homozygotes and heterozygotes with wild homozygotes (i.e., AA versus GG [OR_1] and AG versus GG [OR_2] in the *HTRA1* rs11200638 G→A polymorphism). We estimated and characterized the prevalence of the risk allele with only the data from controls. When we analyzed genotype data in the meta-analysis, zero cell counts were assigned a fixed value (typically 0.5). In addition, we calculated the population attributable risk (PAR) of the risk allele according to the Chang et al. [112] method.

We first compared the alleles for cases and controls to detect overall differences and genetic association. Allele frequencies were computed for studies reporting only genotypic data. Pooled odds ratios were computed two times: by the fixed effects model of Mantel and Haenszel [113], and by the random effects model of DerSimonian and Laird [114]. Random effects incorporated an estimate of between-study variance and provided wider confidence intervals when the results of the constituent studies differed. The random effects model was more appropriate when heterogeneity was present [115]. Unless otherwise stated, the random effects estimates reported here were calculated by the DerSimonian and Laird model.

Our primary genetic analysis of the *HTRA1* rs11200638 G→A polymorphism, the *LOC387715/ARMS2* rs10490924

G-to -T polymorphism, and AMD was based on the comparisons between risk-variant homozygotes and heterozygotes versus wild homozygotes so that the strength of the genetic association and the genetic mode of action could be identified exactly. Once an overall gene effect was confirmed, the genotype effects and genetic model were estimated by using the genetic model-free approach suggested by Minelli et al. [116], in which no assumptions about genetic models are required. A multivariate meta-analysis employing the Bayesian method [116] was used to calculate OR_1 and OR_2. The logarithm (log) odds ratios were modeled on the basis of both between- and within-study variations. A stochastic parameter lambda (λ), equal to the ratio of log OR_2 and log OR_1, was also computed [115]. The parameter λ suggested the genetic mode of action; specifically, the model is a recessive model if $\lambda=0$, a codominant model if $\lambda=0.5$, a dominant model if $\lambda=1$, and homozygous or heterosis model if $\lambda<0$ or $\lambda>1$.

We examined the deviations from the HWE in control populations for each study by using the exact method [117]. For all the analyses, we compared results between inclusion and exclusion of studies in Hardy–Weinberg (HW) disequilibrium. In addition, all studies were included regardless of HWE and provided a revision of the degree of HW disequilibrium by using the inbreeding coefficient (F) suggested by Trikalinos et al. [118]. In brief, data in the control group were used to assess the F value for each study. Predicted genotype frequencies were estimated and then used to replace the observed frequencies in the summary analysis of magnitude and the genetic model.

In sensitivity analysis, we estimated between-study heterogeneity across all eligible comparisons using Cochran's Q statistic [115]. We also reported the I^2 statistic, which describes the percentage of variability in point estimates due to sample heterogeneity rather than sampling error [119, 120], and can quantify heterogeneity irrespective of the number of studies [120,121]. I^2 values larger than 75% were considered to represent a "notable" heterogeneity [120,121]. Publication bias among studies was assessed by funnel plots [122] and cumulative meta-analysis [123]. In the analysis of subgroups, we estimated odds ratios according to racial descent (Caucasians versus East Asians) and AMD type (wet AMD and other subtype or combined AMD).

All analyses were conducted with Stata software, version 9.0 (StataCorp, 2005) [124], using the *meta, metan, metabias, metacum, and metareg* commands, except the Bayesian method of genotype-based analysis. We fitted the Bayesian models by using Markov chain Monte Carlo methods with a Bayesian framework and performed our inferences using WinBUGS 1.4.3 (Imperial College School of Medicine at St Mary's, London 2003) [125], taking advantage of its flexibility as well as its ability to incorporate full uncertainty across all unknown parameters. Bayesian analyses yielded

credible intervals rather than confidence intervals; a 95% credible interval (*CrI*) describes a range in which it is probable that an unknown quantity lies within this interval. A "burn-in" of 10,000 iterations is performed for models, followed by 50,000 iterations for parameter estimates. A p value less than 0.05 was considered statistically significant.

RESULTS

Eligible studies: A total of 29 studies were identified based on our search strategies, of which 13 studies [95-106,108] were eligible for inclusion in this meta-analysis; all of these were written in English. One [101] did not report genotype information in their paper, but online supporting materials provided the data. Two of the studies [100,106] did not have genotypic data, but the authors kindly sent the supplementary information to us. Sixteen studies were ineligible for the following reasons: six were reviews [22,24,126-129], six did not study the association between the *HTRA1* rs11200638 G→A polymorphism and AMD [92-94,130-132], two [90, 91] were duplicated reports of the most recent and comprehensive one [97], and one did not have genotype data [107].

Detailed characteristics of the 13 included studies on the association between *HTRA1* rs11200638 G→A polymorphism and AMD are presented in Table 1. Among them, six studies related to Caucasian subjects, six to East Asians, and one to Indians. The average age of subjects ranged from 64.0 years to 81.2 years for cases and from 64.0 years to 77.4 years for controls. Characteristics of the 13 included studies on the association between *LOC387715/ARMS2* rs10490924 G→T polymorphism and AMD are presented in Table 2. Among them, 14 studies related to Caucasians, three to East Asians, and one to Indians. The average age ranged from 60 to 79 years for cases and from 60 to 77 years for controls. All of the eligible studies had case-control designs. Cases in the studies were recruited from hospital patients and controls were mainly healthy populations recruited from the hospital or community and unrelated to cases.

Allele comparison: Data from the control groups were used to calculate the summary allele frequency. The frequency of the risk allele A in the *HTRA1* rs11200638 G→A polymorphism among controls was 32.33% (95% confidence interval [*CI*]: 26.29, 38.38), and was significantly higher in Asians than in Caucasians (40.11% [95% *CI*: 35.11, 45.12] versus 23.25% [95% *CI*: 18.41, 28.09], p=0.0001). The frequency of the risk allele T in the *LOC387715/ARMS2* rs10490924 G→T polymorphism among controls was 25.17% (95% *CI*: 17.33, 33.00), and was also significantly higher in Asians than in Caucasians (38.67% [95% *CI*: 34.63, 42.71] versus 21.62% [95% *CI*: 17.41, 28.83], p=0.0000178).

All of the 13 studies were included to evaluate the association between the *HTRA1* rs11200638 G→A polymorphism and AMD [95-106,108]. As shown in Figure

1A, individuals with the A allele experienced a 2.80-fold increased risk of AMD when compared to individuals with the G allele (random effect *OR*=2.910, 95% *CI*: 2.552, 3.318; *Q*=25.769, p=0.012, I^2=53.4%). The magnitude of the effect was similar for Asians (random effect *OR*=2.841, 95% *CI*: 2.482, 3.252) and Caucasians (random effect *OR*=2.981, 95% *CI*: 2.357, 3.370). However, there was significantly greater between-study heterogeneity among Caucasians (*Q*=20.128, p=0.001, I^2=75.2%) than Asians (*Q*=5.636, p=0.465, I^2=0.0%). Excluding and adjusting two studies [96,97] with Hardy–Weinberg equilibrium did not change the results (data not shown). After appropriately carrying out a set of prespecified subgroups [97], a low level of between-study heterogeneity was found (random effect *OR*=3.043, 95% *CI*: 2.725, 3.397; *Q*=14.318, p=0.216, I^2=23.2%). We did not find any evidence of publication bias in the eligible studies (corrected Begg's test *z*=0.43, corrected p=0.669). Figure 2 shows the cumulative meta-analysis results; they remained significant and were consistent over time.

The association between the *LOC387715/ ARMS2* rs10490924 G→T polymorphism and AMD was also evaluated. As shown in Figure 1B, individuals with the T allele had a 2.734 fold increased risk of AMD when compared to individuals with the G allele (random effect *OR*=2.734, 95% *CI*: 2.366, 3.158; *Q*=80.195, p=0.000, I^2=78.8%). The magnitude of the effect was similar between Asians (random effect *OR*=2.692, 95% *CI*: 2.086, 3.315) and Caucasians (random effect *OR*=2.794, 95% *CI*: 2.333, 3.346). There was also a significant difference between-study heterogeneity among Caucasians (*Q*=73.265, p=0.000, I^2=83.6%) as opposed to Asians (*Q*=0.481, p=0.786, I^2=0.0%). Figure 3 shows the cumulative meta-analysis results; they remained significant and were consistent over time.

Genotype comparison: The genotype frequency of the *HTRA1* rs11200638 G→A polymorphism between case and control groups is presented in Table 3. The genotype effects for AA versus GG (*OR*₁) and AG versus GG (*OR*₂) were calculated for each study. The genotype frequency of the *LOC387715/ARMS2* rs10490924 G→T polymorphism between the case and control groups is presented in Table 4. The genotype effects for TT versus GG (*OR*₁) and TG versus GG (*OR*₂) were calculated for each study.

In our primary analysis, multivariate meta-analysis was conducted to estimate the pooled risk and there was a significantly increased risk of AMD among individuals with both homozygous variant AA genotype (Bayesian random effect *OR*₁=8.469, 95% *CrI*: 6.766, 10.710) and heterozygous variant AG genotype (Bayesian random effect *OR*₂=2.243, 95% *CrI*: 1.969, 2.559) of the *HTRA1* rs11200638 G→A polymorphism. A moderate level of between-study heterogeneity (*Q*=19.201, p=0.084, I^2=37.5%) was found for the homozygous AA genotype and no between-study heterogeneity (*Q*=13.951, p=0.304, I^2=14.0%) was found for

TABLE 1. CHARACTERISTICS OF CASE-CONTROL STUDIES INCLUDED IN A META-ANALYSIS OF THE ASSOCIATION BETWEEN THE *HTRA1* GENE POLYMORPHISMS AND AMD

Ref	Year	Region, country study was conducted	Ethnicity	Study design	Sex composition in cases (% males)	Mean age (years)		Cases	Controls	Number of eligible subjects	
						Cases	Controls			Cases	Controls
[101]	2006	China	East Asian	Case-control	68	74.9	74.2	Wet AMD	Age matched controls without AMD, confirmed by full ophthalmologic examination	96	130
[98]	2007	Austria	Caucasian	Case-control	35.5	78	77.4	Exudative AMD in AMD level 4	Caucasians without AMD on the base of a detailed eye examination and fundus examination	242	157
[102]	2007	China	East Asian	Case-control	45.1	64.0/	64	Drusen, and wet AMD	Without any AMD, confirmed by a normal eye examination	164	106
[105]	2007	Japan	East Asian	Case-control	72.4	71.9	67.9	AMD, combined	Without AMD and unrelated to cases, confirmed by full ophthalmologic examination	123	133
[106]	2007	Japan	East Asian	Case-control	79.5	75.7	71.2	Wet AMD	Hospital-based controls without retinal diseases and AMD on the base of full ophthalmologic examination	73	94
[95]	2007	USA	Caucasian	Case-control	NR	>68.0	>68.0	AMD, combined	Without AMD on the base of full ophthalmologic examination	535	288
[99]	2007	France	Caucasian	Case-control	NR	>65.0	>65.0	Exudative AMD	Without any type of drusen, geographic atrophy, or exudative AMD.	200	116
[96]	2007	USA	Caucasian	Case-control	NR	71.3	72.8	Wet AMD	Without AMD on the base of full ophthalmologic examination	134	134
[100]	2007	UK	Caucasian	Case-control	40.6	>65.0	>65.0	Wet AMD	Without AMD on the base of full ophthalmologic examination	401	266
[97]	2008	USA	Caucasian	Case-control	49.0/ 52.5/ 38.0/ 44.4	81.2/ 78.9/ 81.0/ 78.3	74	bilateral wet AMD, unilateral wet AMD, bilateral GA, and unilateral GA.	Without any type of drusen, GA, AMD, and RPE	776	294
[103]	2008	China	East Asian	Case-control	54	71.2	71.5	Dry and wet AMD	Age and sex matched controls without any visual impairment, excluded a family history of AMD and any type of drusen, geographic atrophy, CNV, or other retinal disorder in either eye.	95	90
[104]	2008	China	East Asian	Case-control	54	75.5	73.3	Exudative AMD	Without any AMD and any other major eye diseases	163	183
[108]	2008	India	Indian Asian	Case-control	NR	68.8	64.4	AMD, combined	Ethnic matched controls, without a family history of AMD or any other ocular or systemic diseases	250	250

TABLE 2. CHARACTERISTICS OF CASE-CONTROL STUDIES INCLUDED IN A META-ANALYSIS OF THE ASSOCIATION BETWEEN THE *LOC387715* GENE POLYMORPHISMS AND AMD

| Ref	Year	Region, country study was conducted	Ethnicity	Study design composition in cases (% males)	Sex	Mean age (years)		Cases	Controls	Number of eligible subjects	
						Cases	Controls			Cases	Controls
[106]	2007	Japan	East Asian	Case-control	79.5	75.7	71.2	Wet AMD	Hospital-based controls without retinal diseases and AMD on the base of full ophthalmologic examination	73	94
[95]	2007	USA	Caucasian	Case-control	NR	>68.0	>68.0	Wet AMD+Dry AMD	Without AMD on the base of full ophthalmologic examination	431	280
[99]	2007	France	Caucasian	Case-control	NR	>65.0	>65.0	Wet AMD	Without any type of drusen, geographic atrophy, or exudative AMD.	118	116
[96]	2007	USA	Caucasian	Case-control	NR	71.3	72.8	Wet AMD	Without AMD on the base of full ophthalmologic examination	134	134
[100]	2007	UK	Caucasian	Case-control	40.6	>65.0	>65.0	Wet AMD	Without AMD on the base of full ophthalmologic examination	401	266
[108]	2008	India	Indian Asian	Case-control	NR	68.8	64.4	Wet AMD+Dry AMD	Ethnic matched controls, without a family history of AMD or any other ocular or systemic diseases	193	203
[3]	2008	China	East Asian	Case-control	58.7	66	66.1	Wet AMD	Without any AMD and any other major eye diseases aside from mild age-related cataracts	121	132
[84]	2005	Germany	Caucasian	Case-control	NR	NR	NR	Wet AMD+Dry AMD	Without any AMD and any other major eye diseases	759	594
[84]	2005	Germany	Caucasian	Case-control	35.1	75.01	68.25	Wet AMD+Dry AMD	Unrelated controls without any AMD and any other major eye diseases	361	328
[133]	2006	USA	Caucasian	Case-control	42	79.5	76.5	Wet AMD+Dry AMD	Without AMD on the base of full ophthalmologic examination	693	172
[133]	2006	USA	Mixed	Case-control	44	73.2	70.3	Wet AMD+Dry AMD	Without AMD on the base of full ophthalmologic examination	120	995
[56]	2007	Russia	Caucasian	Case-control	27.7	72.6	71.1	Wet AMD+Dry AMD	Free of macular changes	155	151
??	2007	Japan	East Asian	Case-control	70.5	73.4	73.6	Wet AMD	Without any AMD	95	99
??	2007	USA	Caucasian	Nested case-control	35.2	60.1	60.2	Wet AMD+Dry AMD	Within 1 year of the same age with cases, and underwent eye examination in the past 2 years	445	1041
[128]	2007	USA	Caucasian	Case-control	42.6	79	72	Wet AMD+Dry AMD	AMD free controls	399	329
[13]	2007	Australia	Caucasian	Cohort	39.9	75.6	74.9	Wet AMD+Dry AMD	AMD free controls	278	557
[93]	2007	USA	Caucasian	Case-control	NR	NR	NR	Wet AMD	Without any AMD	87	232
[83]	2008	USA	Caucasian	Case-control	39.6	79.1	72.9	Wet AMD+Dry AMD	Without AMD on the base of full ophthalmologic examination	164	155

A

B
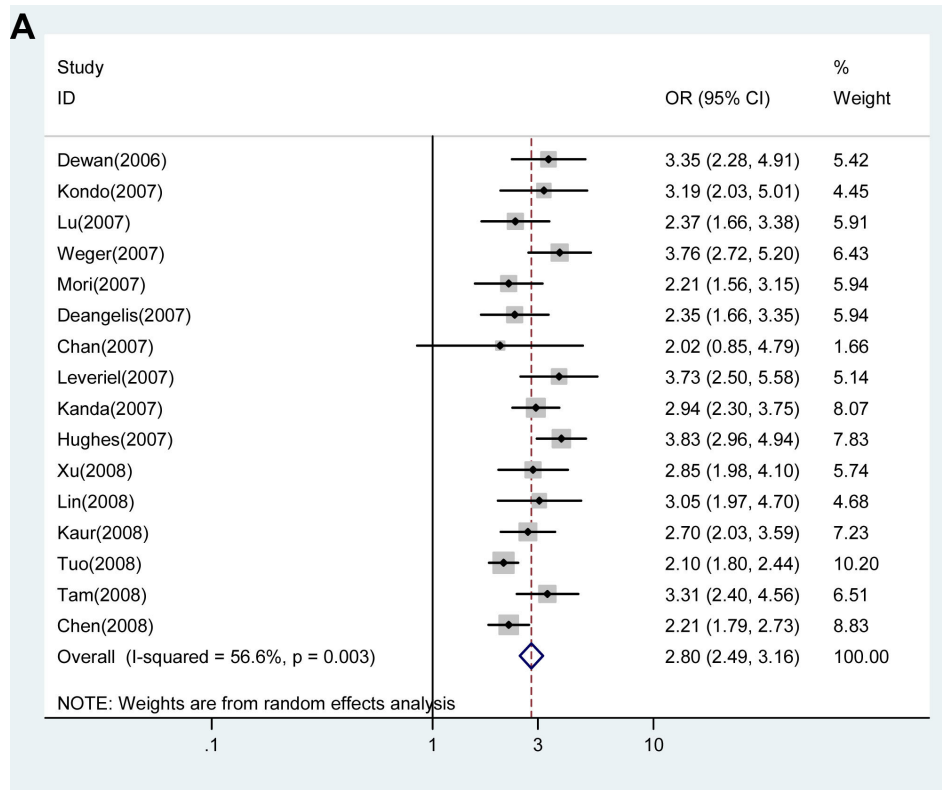
Figure 1. Random-effects meta-analysis of allele (A versus G) of the HTRA1 gene rs11200638 G→A polymorphism and age related macular degeneration (AMD). **A**: Results from random-effects meta-analysis of allele (A versus G) of the *HTRA1* gene rs11200638 G→A polymorphism and AMD. **B**: Results of the random-effects meta-analysis of the allele (T versus G) of the *LOC387715/ARMS2* gene rs10490924 G→T polymorphism and AMD. Reference numbers are given in parentheses. For study details, see Table 1 and Table 2.
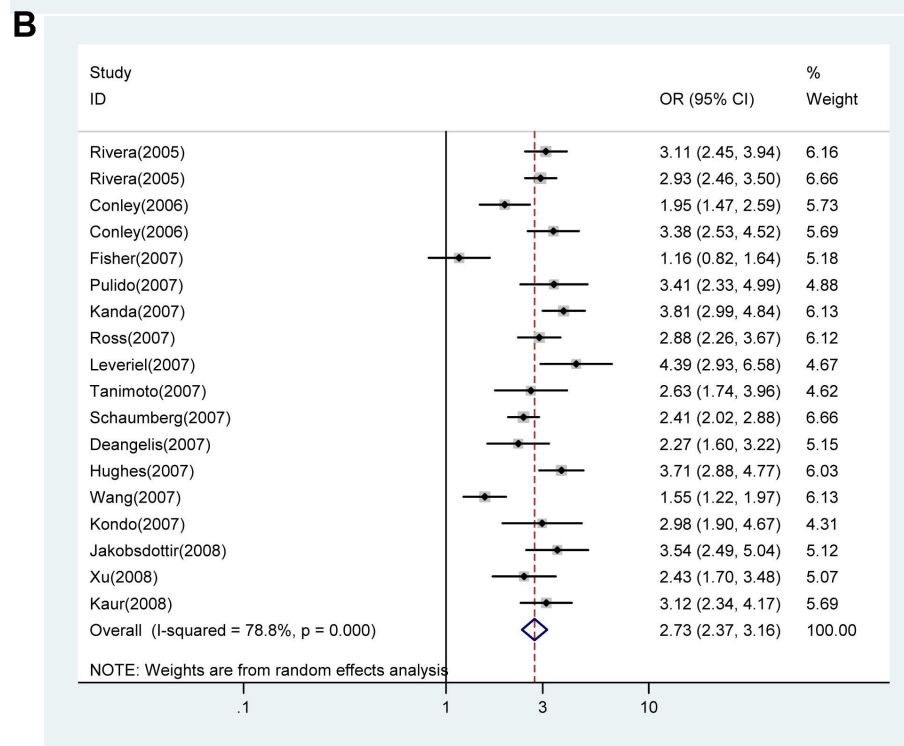
the heterozygous AG genotype. The estimated parameter λ was 0.378 (95% *CrI*: 0.329, 0.428), which suggested a moderate codominant genetic mode of action. When we removed the two studies [96,97] with HW disequilibrium, similar results appeared with the pooled OR_1, OR_2, and λ of 9.257 (95% *CrI*: 7.267, 11.910), 2.334 (95% *CrI*: 2.012, 2.706), and 0.380 (95% *CrI*: 0.327, 0.435), respectively; however, no significant between-study heterogeneity was
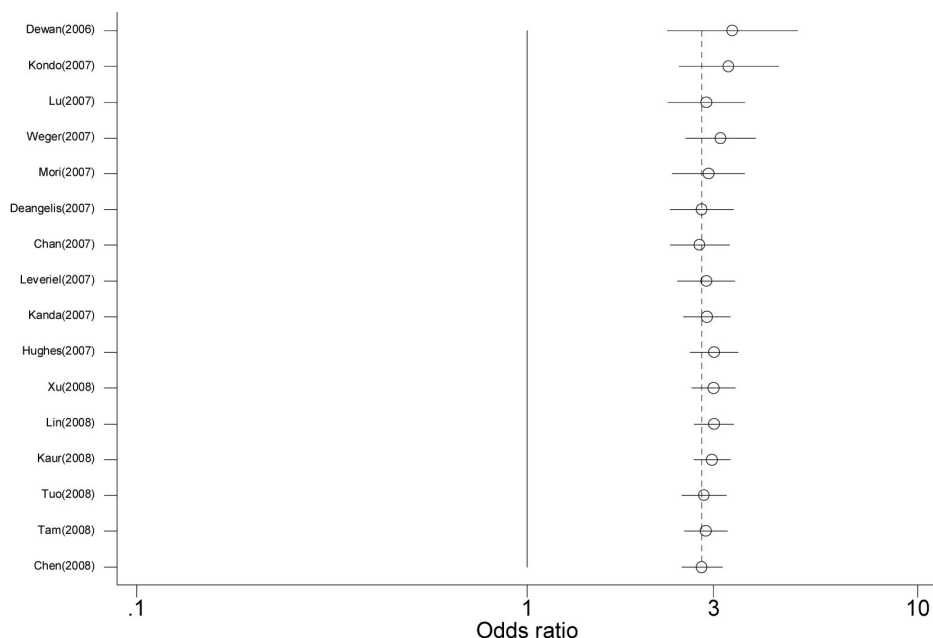
Figure 2. Cumulative random-effects meta-analysis of allele (A versus G) of the *HTRA1* gene rs11200638 G→A polymorphism and age related macular degeneration (AMD). For study details, see Table 3.
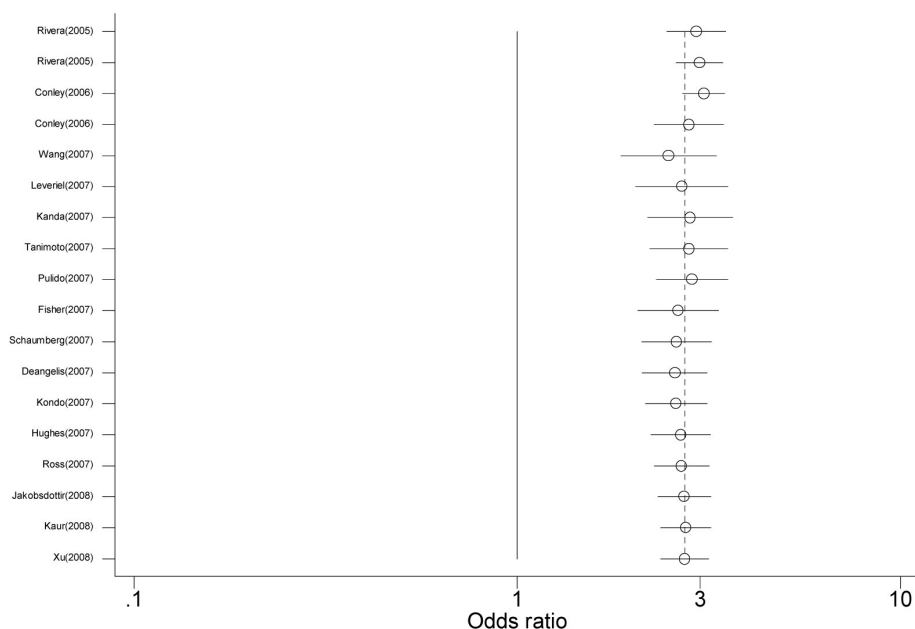

Figure 3. Cumulative random-effects meta-analysis of allele (G versus T) of the *LOC387715/ ARMS2* gene rs10490924 G→T polymorphism and age related macular degeneration (AMD). For study details, see Table 4.

found for either the homozygous AA genotype (Q=13.898, p=0.178, I^2=28.0%) or the heterozygous AG genotype (Q=13.041, p=0.221, I^2=23.3%). The pooled estimates also remained similar after adjusting HW disequilibrium by coefficient F (OR_1=9.065 [95% *CrI*: 7.397, 11.180], OR_2=2.306 [95% *CrI*: 2.039, 2.607], and λ=0.379 [95% *CrI*: 0.332, 0.427]).

Multivariate meta-analysis also showed that there was a significantly increased risk of AMD among individuals with both the homozygous variant TT genotype (Bayesian random effect OR_1=7.512, 95% *CrI*: 5.703, 9.659) and heterozygous variant TG genotype (Bayesian random effect OR_2=2.353, 95% *CrI*: 2.072, 2.665) of the *LOC387715/ ARMS2* rs10490924 G→T polymorphism. The estimated parameter for λ was 0.426 (95% *CrI*: 0.387, 0.467), which suggested a moderate codominant genetic mode of action.

For the *HTRA1* rs11200638 G→A polymorphism, stratification by ethnicity indicated a considerable variation in

TABLE 3. THE ASSOCIATION BETWEEN THE *HTRA1* GENE POLYMORPHISMS AND AMD— ALLELE AND GENOTYPE FREQUENCIES OF CASE-CONTROL STUDIES INCLUDED IN A META-ANALYSIS

| | | Genotype distribution | | | | | | | | | | | AA/GG | | AG/GG | | A/G | |
| | | cases | | | | | controls | | | | | | | | | | | |
Ref	Year	N	AA	AG	GG	P value for HWE	N	AA	AG	GG	P value for HWE	OR1	95% CI	OR2	95% CI	OR	95% CI
[101]	2006	96	44	40	18	0.266	130	14	59	57	0.976	9.952	4.465~22.184	2.147	1.104~4.174	3.626	2.450~5.368
[98]	2007	242	67	108	67	0.247	157	8	50	99	0.877	12.375	5.583~27.432	3.192	2.022~5.039	3.723	2.693~5.158
[102]	2007	164	68	77	19	0.924	106	15	63	28	0.104	6.681	2.980~14.979	1.801	0.921~3.523	2.37	1.664~3.375
[105]	2007	123	45	55	26	0.488	133	22	57	54	0.582	4.248	2.127~8.487	2.004	1.103~3.640	2.231	1.566~3.178
[106]	2007	73	29	39	5	0.239	94	16	40	38	0.627	13.775	4.520~41.984	7.41	2.642~20.786	3.189	2.029~5.013
[95]	2007	457	102	183	172	<0.001	280	11	90	179	0.997	9.65	5.006~18.601	2.112	1.525~2.937	2.937	2.299~3.753
[99]	2007	118	32	57	29	0.937	116	5	41	70	0.948	15.448	5.476~43.582	3.356	1.860~6.055	3.734	2.498~5.583
[96]*	2007	134	43	54	37	0.0837	134	21	43	70	0.0111	3.874	2.009~7.469	2.376	1.350~4.180	2.358	1.657~3.347
[100]	2007	401	106	172	123	0.019	266	6	91	169	0.296	24.274	10.327~57.057	2.597	1.841~3.664	3.826	2.963~4.942
[97]*	2008	776	131	400	245	0.327	294	10	128	156	0.0282	8.341	4.253~16.360	1.99	1.500~2.640	2.042	1.652~2.525
[103]	2008	95	53	33	9	0.53	90	19	47	24	0.903	7.439	2.940~18.819	1.872	0.772~4.541	3.046	1.973~4.703
[104]	2008	163	94	51	18	0.0379	183	38	90	55	0.994	7.559	3.398~14.509	1.732	0.919~3.262	3.31	2.403~4.559
[108]	2008	229	90	89	50	0.0111	184	21	85	78	0.956	6.686	3.695~12.098	1.633	1.028~2.595	2.701	2.033~3.589
Total		3071					2167										

* Hardy–Weinberg disequilibrium in case and/or control group

TABLE 4. THE ASSOCIATION BETWEEN THE *LOC387715* GENE POLYMORPHISMS AND AMD—ALLELE AND GENOTYPE FREQUENCIES OF CASE-CONTROL STUDIES INCLUDED IN A META-ANALYSIS

Genotype distribution

Ref	Year	cases					controls					TT/GG		TG/GG		T/G	
		N	TT	TG	GG	P value for HWE	N	TT	TG	GG	P value for HWE	OR1	95% CI	OR2	95% CI	OR	95% CI
[106]	2007	73	27	40	6	0.25	94	15	41	38	0.783	11.4	3.920~33.155	6.179	2.354~16.217	2.979	1.901~4.668
[95]	2007	431	133	180	118	<0.001	280	12	99	169	0.992	15.875	8.405~29.979	2.604	1.854~3.658	3.809	2.995~4.845
[99]	2008	118	37	55	26	0.811	116	5	40	71	0.978	20.208	7.169~56.962	3.755	2.048~6.886	4.388	2.928~6.576
[96]	2008	134	45	51	38	0.0234	134	22	44	68	0.013	3.66	1.918~6.985	2.074	1.178~3.653	2.271	1.600~3.223
[100]	2005	401	111	170	120	0.00992	266	10	89	167	0.908	15.448	7.761~30.746	2.658	1.878~3.763	3.71	2.884~4.774
[108]	2005	193	81	77	35	0.101	203	25	89	89	0.932	8.239	4.544~14.937	2.2	1.340~3.613	3.123	2.336~4.175
[3]	2006	121	54	49	18	0.472	132	22	70	40	0.651	5.455	2.589~11.491	1.556	0.800~3.026	2.43	1.697~3.480
[84]	2006	759	142	349	268	0.327	594	27	179	388	0.558	7.614	4.904~11.822	2.823	2.225~3.582	2.932	2.459~3.495
[84]	2007	361	88	156	117	0.0471	328	16	109	203	0.962	9.543	5.347~17.030	2.483	1.778~3.468	3.109	2.453~3.940
[133]	2007	693	135	341	217	0.999	172	4	57	111	0.567	17.264	6.223~47.893	3.06	2.131~4.395	1.949	1.466~2.592
[133]	2007	120	18	49	53	0.501	995	43	351	601	0.654	4.747	2.559~8.804	1.583	1.050~2.386	3.384	2.532~4.523
[56]	2007	155	16	66	73	0.982	151	10	66	75	0.669	1.644	0.700~3.859	1.027	0.643~1.643	1.161	0.821~1.641
??	2007	95	39	34	22	0.0398	99	10	50	39	0.58	6.941	2.898~16.491	1.205	0.610~2.380	2.626	1.742~3.958
??	2007	445	68	182	195	0.0694	1041	41	308	692	0.661	5.886	3.872~8.948	2.097	1.645~2.673	2.412	2.023~2.876
[128]	2007	399	69	182	148	0.601	329	12	100	217	0.994	8.431	4.412~16.112	2.669	1.935~3.679	2.883	2.265~3.670
[13]	2007	278	14	120	144	0.216	557	16	179	362	0.547	2.2	1.047~4.623	1.685	1.247~2.278	1.552	1.221~1.974
[93]	2007	87	19	38	30	0.578	232	13	60	159	0.092	7.746	3.459~17.346	3.357	1.911~5.896	3.409	2.331~4.986
[83]	2008	164	40	74	50	0.483	155	10	42	103	0.108	8.24	3.812~17.813	3.63	2.185~6.029	3.54	2.488~5.038
Total		5027					5878										

the size of effects between Asian populations (Bayesian random effect OR_1=7.100, 95% *CrI*: 5.325, 9.494; Bayesian random effect OR_2=2.009, 95% *CrI*: 1.625, 2.511; λ=0.356, 95% *CrI*: 0.267, 0.442) and Caucasian populations (Bayesian random effect OR_1=10.130, 95% *CrI*: 6.323, 0.574; Bayesian random effect OR_2=2.347, 95% *CrI*: 1.918, 2.910; λ=0.368, 95% *CrI*: 0.307, 0.434). A moderate degree of between-study heterogeneity was found for the AA homozygous genotype among both Asians (Q=13.978, p=0.030, I^2=57.1%) and Caucasians (Q=13.203, p=0.022, I^2=62.1%), but no significant between-study heterogeneity was found for the AG homozygous genotype among either population (Asians: Q=7.309, p=0.293, I^2=17.93%; Caucasians: Q=5.165, p=0.396, I^2=3.2%). For the *LOC387715/ ARMS2* rs10490924 G→T polymorphism, a moderate level of between-study heterogeneity was found for the TT homozygous genotype among Caucasians (Q=45.035, p=0.000, I^2=73.8%) and for the TG heterozygous genotype among both Asians (Q=7.783, p=0.020, I^2=74.5%) and Caucasians (Q=29.790, p=0.003, I^2=59.7%); however, no significant degree of between-study heterogeneity was found for the TT homozygous genotype among Asians (Q=1.232, p=0.54, I^2=0.0%).

Results of metaregression analysis indicated that classification of AMD (wet AMD versus combined AMD) was significantly associated with log OR_2 (metaregression beta coefficient=-0.325, p=0.016). We performed stratification analysis on wet AMD and the combined AMD of the *HTRA1* rs11200638 G→A polymorphism, and found a considerable difference in effects between wet AMD (Bayesian random effect OR_1=10.110, 95% *CrI*: 6.998, 16.490; Bayesian random effect OR_2=2.647, 95% *CrI*: 2.132, 3.280; λ=0.420, 95% *CrI*: 0.0.350, 0.491) and combined AMD (Bayesian random effect OR_1=7.087, 95% *CrI*: 5.284, 9.523; Bayesian random effect OR_2=1.931, 95% *CrI*: 1.643, 2.277; λ=0.337, 95% *CrI*: 0.267, 0.408). This stratification exhibited no between-study heterogeneity for either OR_1 (Q=3.232, p=0.664, I^2=0.0%) or OR_2 (Q=0.890, p=0.971, I^2=0.0%) for combined AMD, and found a moderate degree of between-study heterogeneity for OR_1 (Q=13.978, p=0.030, I^2=57.1%) and non-significant between-study heterogeneity for OR_2 (Q=7.309, p=0.293, I^2=17.9%) of the wet AMD (Table 5).

We also performed stratification analysis on the wet AMD and combined AMD of the *LOC387715/ARMS2* rs10490924 G→T polymorphism, and found a considerable difference in effect between wet AMD (Bayesian random effect OR_1=8.567, 95% *CrI*: 5.509, 12.600; Bayesian random effect OR_2=2.519, 95% *CrI*: 1.983, 3.147; λ=0.433, 95% *CrI*: 0.354, 0.513) and combined AMD (Bayesian random effect OR_1=7.021, 95% *CrI*: 7.021; Bayesian random effect OR_2=2.285, 95% *CrI*: 1.921, 2.694; λ=0.428, 95% *CrI*: 0.382, 0.475). This stratification found no between-study

heterogeneity for either OR_1 (Q=5.391, p=0.612, I^2=0.0%) or OR_2 (Q=1.024, p=0.994, I^2=0.0%) for combined AMD, and found a moderate degree of between-study heterogeneity for OR_1 (Q=14.147, p=0.049, I^2=51.6%) and non-significant between-study heterogeneity for OR_2 (Q=7.311, p=0.397, I^2=4.3%) of the wet AMD (Table 5).

There was no evidence of small study bias or publication bias for the two comparisons. For the *HTRA1* rs11200638 G→A polymorphism, funnel plots for the comparisons made for the AA homozygotes and AG heterozygotes gave corrected p=0.077 (corrected Begg's z=1.77) and corrected p=0.669 (corrected Begg's z=0.43), respectively. Figure 4 shows the cumulative result of meta-analysis of the AA homozygotes and AG heterozygotes; they remained significant and stayed relatively unchanged after the third study (Figure 4A,B). Figure 5 shows the cumulative result of meta-analysis of the TT homozygotes and TG heterozygotes of *LOC387715/ARMS2* rs10490924 with G→T polymorphism; they remained significant and relatively unchanged after the third study.

DISCUSSION

To our knowledge, this is the first general overview of the association between the *HTRA1* rs11200638 G→A polymorphism, the *LOC387715/ARMS2* rs10490924 G→T polymorphism, and susceptibility to AMD. The results of our meta-analysis suggest a strong association and a moderate codominant genetic mode of action. Our primary analysis shows that, for the *HTRA1* rs11200638 G→A polymorphism, the AA homozygotes carry an 8.5 fold increased risk of AMD, and the AG heterozygous variants carry just a 2.5 fold increase in risk when compared with GG homozygotes; for the *LOC387715/ARMS2* rs10490924 G→T polymorphism, the TT homozygotes carry a 7.5 fold increased risk of AMD, and the TG heterozygous variants carry just a 2.4 fold increase in risk when compared with the GG homozygotes. In addition, our allele-based analysis suggests a nearly 3.0-fold increase in susceptibility to AMD among persons with the A allele of the *HTRA1* rs11200638 G→A polymorphism and the T allele of the *LOC387715/ARMS2* rs10490924 G→T polymorphism.

Our findings were based on several gene-association studies, which include several thousand participants and were robust in terms of all the planned and performed sensitivity analyses. We found no evidence of publication bias or small study bias by funnel plots and cumulative meta-analysis; moreover, "moderate," "moderate," and "low" degrees of between-study heterogeneity were found in alleles (A versus G), homozygotes (AA versus GG), and heterozygotes (AG versus GG) of the association between the *HTRA1* rs11200638 G→A polymorphism and AMD. When HWE was examined, 11 of the 13 studies showed no deviation and two showed some deviation. The removal of the two HW disequilibrium studies meant that our overall results were also

TABLE 5. AGE RELATED MACULAR DEGENERATION (AMD): *HTRA1* SNPS VERSUS *ARMS2* SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS).

Comparison	No. of studies	Total sample size (n)	Bayesian random effects		Fixed effects		Random effects		Heterogeneity		
			Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Q	P value	I2 (%)
HTRA1 (rs11200638)											
A allele versus G allele											
Total	16	4034/3212	/	/	2.664	2.476, 2.867	2.803	2.486, 3.159	34.576	0.003	56.6
HWE	14	3124/2784	/	/	2.754	2.542, 2.984	2.909	2.547, 3.324	30.471	0.004	57.4
Adjusted HWE	16	4034/3212	/	/	2.701	2.510, 2.907	2.827	2.519, 3.173	31.923	0.007	53
East Asian	7	835/868	/	/	2.847	2.473, 3.278	2.847	2.473, 3.278	4.873	0.56	0
Caucasian	8	2970/2160	/	/	2.589	2.366, 2.833	2.8	2.289, 3.424	28.452	0	75.4
Seven studies with 2 SNPs	7	1533/1206	/	/	3.059	2.717, 3.444	3.053	2.681, 3.478	7.028	0.318	14.7
AA versus GG											
Total	16	4034/3212	7.972	6.453, 9.778	7.21	6.035, 8.614	7.737	6.096, 9.821	24.308	0.06	39.2
HWE	14	3124/2784	8.424	6.667, 10.540	7.515	6.201, 9.107	8.16	6.314, 10.546	20.51	0.083	37.6
Adjusted HWE	16	4034/3212	8.225	6.700, 10.030	7.423	6.205, 8.880	7.928	6.286, 10.000	22.776	0.089	35.3
East Asian	7	835/868	7.604	5.541, 10.100	7.273	5.390, 9.815	7.273	5.390, 9.815	4.27	0.64	0
Caucasian	8	2970/2160	8.691	5.813, 13.600	7.258	5.719, 9.211	8.687	5.556, 13.582	19.969	0.006	65.8
Wet AMD	8	1348/1212	9.484	6.834, 12.800	9.205	6.941, 12.207	9.843	6.539, 14.817	14.147	0.049	51.6
Wet AMD + Dry AMD	8	2686/2000	6.561	5.137, 8.270	6.138	4.881, 7.719	6.138	4.881, 7.719	5.391	0.612	0
Seven studies investigated 2 SNPs	7	1533/1206	8.967	5.964, 12.920	8.534	6.411, 11.360	9.309	5.924, 14.682	14.097	0.029	58.8
AG versus GG											
Total	16	4034/3212	2.226	1.982, 2.496	2.168	1.944, 2.418	2.18	1.943, 2.447	15.784	0.397	5
HWE	14	3124/2784	2.28	2.006, 2.589	2.193	1.943, 2.475	2.232	1.935, 2.574	15.295	0.289	15.1
Adjusted HWE	16	4034/3212	2.252	2.011, 2.516	2.192	1.966, 2.445	2.204	1.962, 2.477	15.942	0.386	5.9
East Asian	7	835/868	2.277	1.781, 2.866	2.202	1.684, 2.878	2.219	1.665, 2.957	6.812	0.339	12.3
Caucasian	8	2970/2160	2.273	1.916, 2.741	2.205	1.948, 2.495	2.221	1.949, 2.531	7.453	0.383	6.1

TABLE 5. CONTINUED.

Comparison	No. of studies	Total sample size (n)	Bayesian random effects		Fixed effects		Random effects		Heterogeneity		
			Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Q	P value	I2 (%)
Wet AMD	8	1348/1212	2.692	2.197, 3.249	2.706	2.231, 3.281	2.708	2.219, 3.305	7.311	0.397	4.3
Wet AMD + Dry AMD	8	2686/2000	1.959	1.723, 2.222	1.953	1.711, 2.230	1.953	1.711, 2.230	1.024	0.994	0
Seven studies with 2 SNPs	7	1533/1206	2.392	1.938, 2.907	2.396	2.004, 2.866	2.471	1.946, 3.138	9.387	0.153	36.2
λ											
Total			0.386	0.343, 0.430							
HWE			0.387	0.340, 0.435							
Adjusted HWE			0.386	0.343, 0.429							
East Asian			0.403	0.311, 0.491							
Caucasian			0.381	0.327, 0.438							
Wet AMD			0.441	0.373, 0.510							
Wet AMD + Dry AMD			0.359	0.300, 0.419							
Seven studies with 2 SNPs			0.4	0.331, 0.471							
LOC387715/ARMS2 (rs10490924) T allele versus G allele											
Total	18	5027/5878	/	/	2.725	2.556, 2.906	2.734	2.366, 3.158	80.195	0	78.8
HWE	17	4893/5744	/	/	2.742	2.569, 2.928	2.761	2.376, 3.209	79.116	0	79.8
Adjusted HWE	18	5027/5878	/	/	2.715	2.547, 2.896	2.719	2.351, 3.145	81.68	0	79.2
East Asian	3	289/325	/	/	2.692	2.086, 3.315	2.692	2.086, 3.315	0.481	0.786	0
Caucasian	13	4425/4355	/	/	2.769	2.580, 2.972	2.794	2.333, 3.346	73.265	0	83.6
Seven studies with 2 SNPs	7	1471/1225	/	/	3.276	2.912, 3.686	3.211	2.711, 3.802	11.596	0.072	48.3

TABLE 5. CONTINUED`

Comparison	No. of studies	Total sample size (n)	Bayesian random effects		Fixed effects		Random effects		Heterogeneity		
			Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Q	P value	I2 (%)
TT versus GG											
Total	18	5027/5878	7.512	5.703, 9.659	7.096	6.069, 8.296	7.216	5.492, 9.480	48.208	0	65.3
HWE	17	4893/5744	7.826	5.886, 10.140	7.394	6.294, 8.683	7.533	5.707, 9.943	43.926	0	64.2
Adjusted HWE	18	5027/5878	7.51	5.692, 9.672	7.1	6.071, 8.303	7.209	5.483, 9.480	48.331	0	65.4
East Asian	3	289/325	/	/	6.934	4.206, 11.431	6.934	4.206, 11.431	1.232	0.54	0
Caucasian	13	4425/4355	7.57	5.326, 10.850	7.261	6.076, 8.677	7.41	5.176, 10.607	45.035	0	73.8
Wet AMD	7	1029/1073	8.567	5.509, 12.600	7.828	5.786, 10.582	8.273	5.191, 13.185	13.738	0.033	56.9
Wet AMD + Dry AMD	11	3998/4805	7.021	4.678, 9.950	6.846	5.703, 8.218	6.708	4.734, 9.505	33.919	0	71.1
Seven studies with 2 SNPs	7	1471/1225	9.767	6.169, 14.480	9.134	6.951, 12.002	9.521	5.922, 15.307	17.209	0.009	65.6
GT versus GG											
Total	18	5027/5878	2.353	2.072, 2.665	2.336	2.134, 2.558	2.324	1.993, 2.709	42.812	0.001	60.3
HWE	17	4893/5744	2.38	2.093, 2.702	2.343	2.138, 2.569	2.336	1.990, 2.741	42.638	0	62.5
Adjusted HWE	18	5027/5878	2.334	2.058, 2.643	2.316	2.115, 2.535	2.29	1.956, 2.681	45.09	0	62.3
East Asian	3	289/325	/	/	1.843	1.203, 2.823	2.119	0.893, 5.029	7.783	0.02	74.5
Caucasian	13	4425/4355	2.424	2.062, 2.865	2.422	2.198, 2.669	2.445	2.082, 2.871	29.79	0.003	59.7
Wet AMD	7	1029/1073	2.519	1.983, 3.147	2.531	2.053, 3.122	2.519	1.813, 3.501	13.05	0.042	54
Wet AMD + Dry AMD	11	3998/4805	2.285	1.921, 2.694	2.293	2.074, 2.536	2.253	1.886, 2.691	29.067	0.001	65.6
Seven studies with 2 SNPs	7	1471/1225	2.507	1.999, 3.088	2.564	2.137, 3.076	2.567	2.065, 3.191	7.834	0.251	23.4
λ											
Total			0.426	0.387, 0.467							
HWE			0.423	0.384, 0.463							
Adjusted HWE			0.422	0.383, 0.462							

TABLE 5. CONTINUED'

Comparison	No. of studies	Total sample size (n)	Bayesian random effects		Fixed effects		Random effects		Heterogeneity		
			Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Q	P value	I2 (%)
East Asian			/	/							
Caucasian			0.438	0.395, 0.483							
Wet AMD			0.433	0.354, 0.513							
Wet AMD + Dry AMD			0.428	0.382, 0.475							
Seven studies with 2 SNPs			0.406	0.341, 0.472							

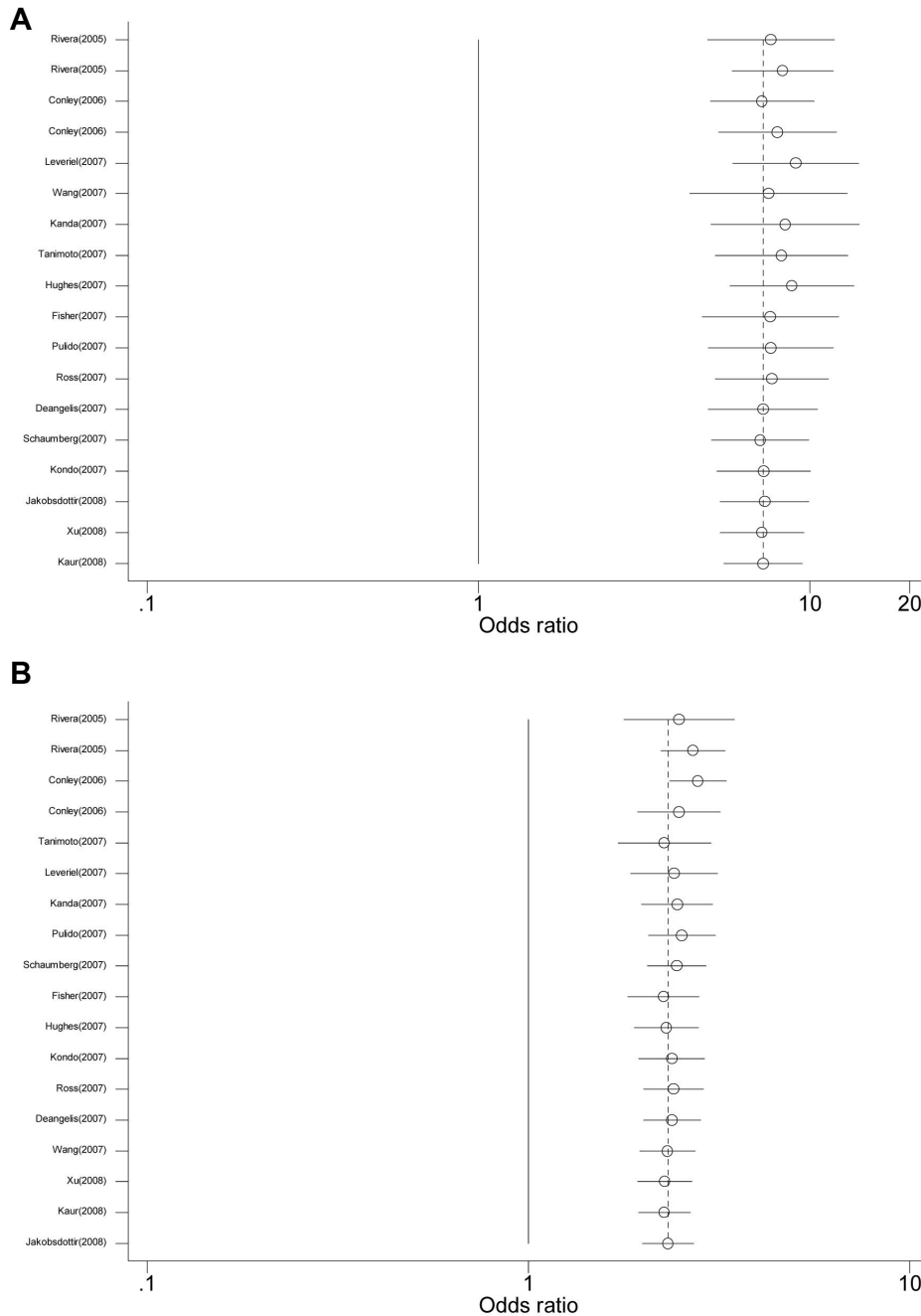Summary odds ratios of *HTRA1* rs11200638 polymorphism and *LOC387715/ARMS2* rs10490924 polymorphism.
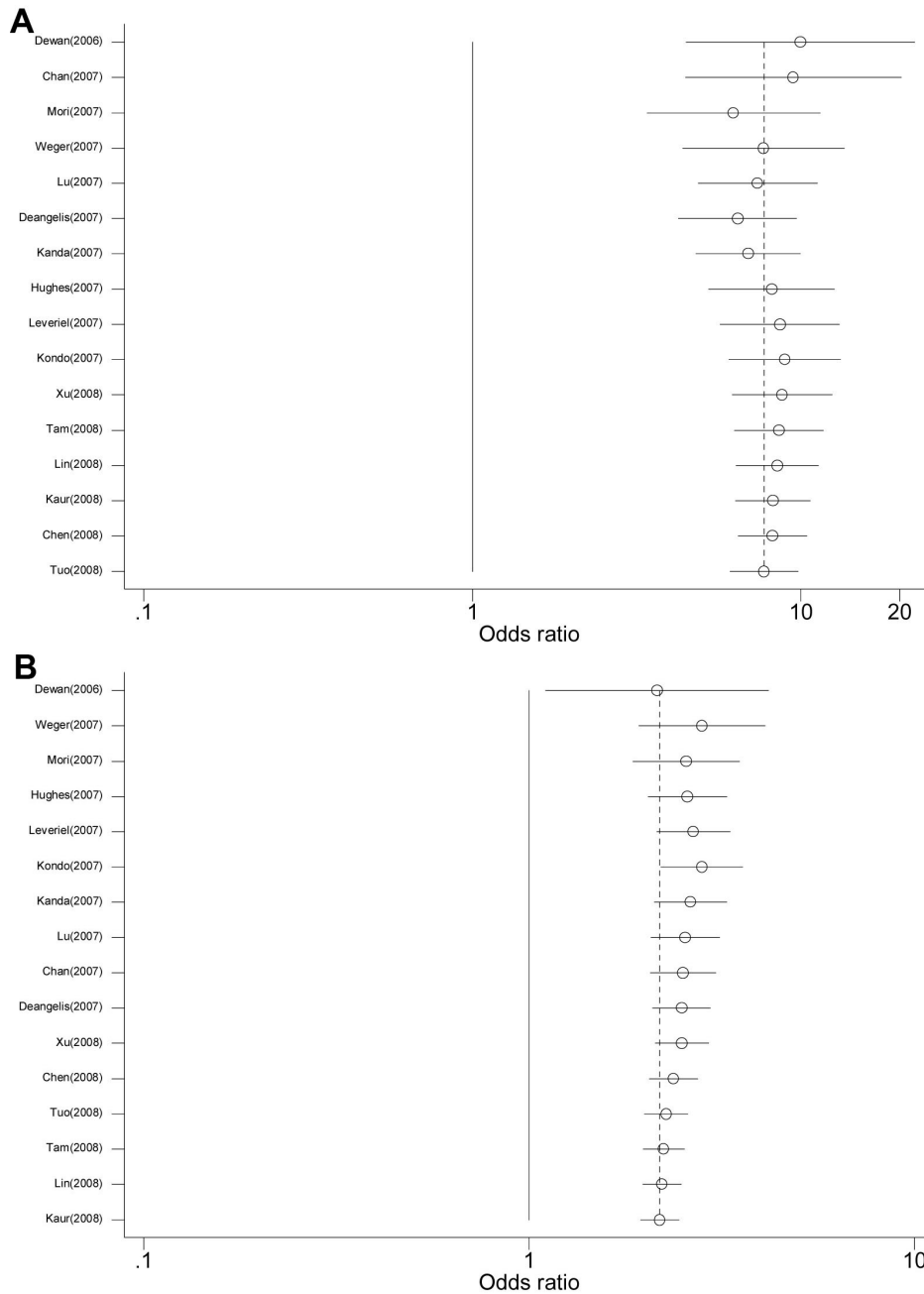
Figure 4. Cumulative random-effects meta-analysis of homozygous (**A**: AA versus GG) and heterozygous (**B**: AG versus GG) genotypes of the *HTRA1* gene rs11200638 G→A polymorphism and ager related macular degeneration (AMD). For study details, see Table 3.

robust; statistical adjustment for the deviations were similar and consistent with the incipient results. The point estimate values were closer to a codominant model after removal of the HW disequilibrium studies and statistical adjustment for the deviation; this suggested a multiplicative genetic mode of action that needs to be verified by more studies, particularly large-scale, long-term longitudinal studies. Moderate between-study heterogeneity was also found in the alleles (T versus G), homozygotes (TT versus GG), and heterozygotes (TG versus GG) of the association between the *LOC387715/ ARMS2* rs10490924 G→T polymorphism and AMD.

However, the data we collected in this systematic review can only support a moderate codominant genetic model with a tight confidence interval.

The *HTRA1* gene encodes a member of the trypsin family of serine proteases [133]. The precise pathomechanism by which the HTRA1 rs11200638 A risk allele affects susceptibility to AMD is still unclear [134,135]. The upregulation of *HTRA1* plays a detrimental role in arthritic disease through its capacity to degrade extracellular matrices (ECMs) directly and to upregulate the expression of matrix metalloproteinase, which results in ECM degradation [88].

A

B

Figure 5. Cumulative random-effects meta-analysis of homozygous (**A**: TT versus GG) and heterozygous (**B**: GT versus GG) genotypes of *LOC387715/ARMS2* gene rs10490924 G→T polymorphism and age related macular degeneration (AMD). For study details, see Table 4.

Yang et al. [90] hypothesized that the most likely mechanism in the involvement of rs11200638 with AMD may be the enhancement of ECM degradation [90]. As shown in the model of laser-induced CNV [136], the destruction of the Bruch membrane leads to CNV development [90]. Although the function of *HTRA1* in ocular tissues is unclear, it is reasonable to speculate that CNV may develop when the Bruch membrane is exposed to the detrimental effects of *HTRA1*. In vitro, higher luciferase expressions have been reported in both ARPE19 and HeLaS3 cells transfected with the *HTRA1* rs1120638 risk homozygote (AA) genotype when compared to the wild-type (GG) [101]. It has been suggested that the presence of the *HTRA1* rs11200638 A risk allele may alter the affinity of transcription factors, including the adaptor-related protein complex 2 alpha and serum response factor to the *HTRA1* promoter [101]. Another potential mechanism by which the *HTRA1* rs11200638 A allele may increase AMD risk is its ability to bind to TGF-β family members and to inhibit signaling of TGF-β family proteins, such as bone morphogenetic protein 2 and bone

morphogenetic protein 4), which have previously been reported to act as negative growth regulators in RPE [89, 137].

Although an association between the *HTRA1* rs11200638 G→A polymorphism and AMD was found, Kanda and others [95] considered that the rs11200638 G→A polymorphism of the *HTRA1* gene did not make a major contribution to regulation of the *HTRA1* gene and there is no association between *HTRA1* G→A polymorphism and AMD. To verify these conclusions, they generated mammalian expression constructs carrying three different lengths of the normal HTRA1 promoter (WT-long, -medium, and -short) and the mutant sequence carrying the AMD-risk allele at the single nucleotide polymorphism (SNP) rs11200638 (SNP-long and -medium), and these constructs were transfected into human embryonic kidney293 (HEK293), human-derived retinal pigment epithelial (ARPE19), and Human retinoblastoma (Y79) cells. As a result, they found that WT and variant SNPs of the *HTRA1* promoter activities did not show significant differences in the luciferase reporter expression, and the WT-short promoter (not including the rs11200638 region) showed higher transcriptional activities than the others. A further quantitative analysis provided no evidence for significant change of mRNA expression between control and AMD retinas. This finding contrasts with the previous original experiment, which suggested an increase in *HTRA1* expression in lymphocytes from AMD patients [90, 127]. Taken together, these studies seem to draw a conflicting conclusion to those of the other studies in our meta-analysis.

Localization of the LOC387715/ARMS2 protein to the mitochondrial outer membrane in transfected mammalian cells suggests intriguing mechanisms through which an A69S change may influence AMD susceptibility. Mitochondria are implicated in the pathogenesis of other age-related neurodegenerative diseases, including Alzheimer disease, Parkinson disease, and so on [138]. Mitochondrial dysfunction associated with aging can result in impairment of the energy metabolism and homeostasis, generation of reactive oxygen species, accumulation of somatic mutations in mitochondrial DNA, and activation of the apoptotic pathway [139-141]. Decreased number and size of mitochondria, loss of cristae, or reduced matrix density are observed in AMD retinas compared with controls, and mitochondrial DNA deletions and cytochrome *c* oxidase-deficient cones accumulate in the aging retina, particularly in the macular region [140,142]. Moreover, mutations in mitochondrial proteins (e.g., dynamin-like guanosine triphosphatase [GTPase] optic atrophy 1 [OPA1]) are associated with optic neurodegenerative disorders [143]. Photoreceptors and RPE contain high levels of polyunsaturated fatty acids and are exposed to intense light and near-arterial levels of oxygen, providing considerable risk for oxidative damage [143,144]. Kanda and others therefore propose that the altered function of the putative mitochondrial

protein LOC387715/ARMS2 by A69S substitution increases the susceptibility to the aging-associated generation of macular photoreceptors [95]. However, they did not observe any significant difference in the expression, stability, or localization of the A69S variant LOC387715/ARMS2 protein in mammalian cells. It is plausible that the A69S alteration modifies the function of the LOC387715/ARMS2 protein by affecting its conformation and/or interaction. For this reason, additional analysis of the LOC387715/ARMS2 protein with Ala or Ser codon 69 and its function in vivo are needed to better understand its contribution to AMD pathogenesis.

Even though the results presented here are contradictory, the A allele of the *HTRA1* gene rs11200638 G→A polymorphism is reasonably common, with an allele frequency of over 30% in a control population and over 40% in an Asian control population, and the T allele frequency of the *LOC387715/ARMS2* rs10490924 G→T polymorphism was 25.17% in a control population and 38.67% in Asians. This means that the effect at the population level, especially for Asian populations, could be quite important. The proportion of AA and AG genotypes of the *HTRA1* rs11200638 G→A polymorphism in a control population is 48% and the pooled OR for these two genotypes is 3.13. These two data were 64.07, 3.47 and 39.81, 3.07 for Asians and Caucasians, respectively. For the *LOC387715/ARMS2* rs10490924 G→T polymorphism, the proportion of TT and GT genotypes in a control population is 38.89% and the pooled *OR* for these two genotypes is 3.05. These two data were 64.00, 3.17 and 35.40, 3.13 for Asians and Caucasians, respectively.

The PAR for the combined genotypes AA and AG of the *HTRA1* rs11200638 G→A polymorphism is 56.0% (63.5% for Asians, 48.4% for Caucasians, 61.3% for wet AMD, 51.1% for combined AMD). The PAR for the combined genotypes TT and GT of *LOC387715/ARMS2* gene rs10490924 G→T polymorphism is 47.9% (55.4% for Asians, 44.1% for Caucasians, 56.8% for wet AMD, 42.4% for combined AMD). In other words, the *HTRA1* rs11200638 G→A polymorphism is involved in over half of all cases of AMD, quite close to the previous estimate of the first major AMD-susceptibility allele, *CFH* Y402H (58.9%) [79]. The *LOC387715/ARMS2* rs10490924 G→T polymorphism is also involved in nearly half of all AMD cases. Higher PAR can explain part of why both these genes (*HTRA1* rs11200638 G→A polymorphism and *LOC387715/ARMS2* rs10490924 G→T polymorphism) play important roles in AMD, especially for wet AMD populations.

In conclusion, this Human Genome Epidemiology (HuGE) systematic review presents strong evidence for an association between the *HTRA1* rs11200638 G→A polymorphism, *LOC387715/ARMS2* rs10490924 G→T polymorphism, and AMD, and suggests that both of these genes play important roles in this disease. Potential gene-gene

and gene-environmental interactions and possible mechanisms of AMD are also summarized and discussed. Our findings suggest that these genetic variations may serve as biomarkers enabling the diagnosis of AMD in a more efficient and economical way. However, large-scale, long-term longitudinal studies are required to substantiate and strengthen this association.

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