High-temperature requirement factor A1 rs11200638 polymorphism and age-related macular degenerative from a comprehensive analysis about 15316 subjects

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Ying Liu, Huipeng Jin, Dong Wei, Wenxiu Li

Ying Liu
Ophthalmic function room

Huipeng Jin
Ophthalmic function room

Dong Wei
Department of Ophthalmology

✉ hgyywd@163.com Corresponding Author
ORCiD: https://orcid.org/0000-0002-0020-5642

Wenxiu Li
Department of Critical Medicine

Prescreen

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**Abstract**

**Background:** The high-temperature requirement factor A1 (HTRA1) gene at the 10q26 locus has been associated with age-related macular degenerative (AMD) risk, with the significantly associated polymorphism being (rs11200638, -625G/A), however, above association is not consistent. We investigated an updated meta-analysis to evaluate the association between rs11200638 polymorphism and AMD risk thoroughly addressing this issue.

**Methods:** An identification was covered with the Pubmed and Chinese Wanfang databases through 27th Jan, 2020. Odds ratios (OR) and 95% confidence intervals (CI) were used to assess the strength of associations. After a thorough and meticulous search, 35 different articles (33 case-control studies with HWE, 22 case-control studies about wet/dry AMD) were retrieved.

**Results:** Individuals carrying A-allele or AA genotype may have an increased risk to be AMD disease. For example, there has a significantly increased relationship between rs11200638 polymorphism and AMD both for Asians (OR: 2.51, 95%CI: 2.22-2.83 for A-allele vs. G-allele) and Caucasians (OR: 2.63, 95%CI: 2.29-3.02 for A-allele vs. G-allele). Moreover, a similar trend in the source of control subgroup was detected. To classify the type of AMD, increased association was also observed in both wet (OR: 3.40, 95%CI: 2.90-3.99 for dominant model) and dry (OR: 2.08, 95%CI: 1.24-3.48 for dominant model) AMD. Finally, based on the different genotyping methods, increased relationships were identified by sequencing, TaqMan, PCR-RFLP and RT-PCR.

**Conclusions:** Our present meta-analysis suggests that the HTRA1 rs11200638 polymorphism are potentially associated with the risk of AMD development, especially about individuals carrying A-allele or AA genotype, who may be as identified targets to detect and intervene in advance. Further studies using larger sample sizes and including information about gene-environment interactions should be conducted to elucidate.

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**Background**

In both developed and developing countries, age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly people [1, 2]. By 2050, the number of people affected by AMD may be estimated to reach 17.8 million [3]. AMD’s visual loss is due to dead/non-functional photoreceptor cells and potential retinal pigment epithelium cells [4]. In clinical practice, AMD is divided into two forms: early dryness that can develop into geographic atrophy (atrophic, non-exudative) and wet (exudative) AMD characterized by choroidal neovascularization (CNV) [5, 6].

Age, race, family history, smoking and sun exposure are common risk factors [7, 8]. Another main factor in the etiology of AMD is genetic susceptibility [9]. A genome-wide association study (GWAS) in 2005 confirmed the association between AMD risk and genetic variations, suggesting that AMD is a polygenic disease [10], and in the following 15 years triggered many studies involving AMD genetic association [11-13]. So far, polymorphism about age-related maculopathy susceptibility 2 (ARMS2) rs10490924, complement factor H (CFH), complement 2 (C2)/complement factor H (CFB), complement component C3 and apolipoprotein E (APOE) haplotypes have been demonstrated as associated factors with susceptibility to AMD [14-18].

As we all known, vascular endothelial growth factor (VEGF) is involved in the development of wet AMD, because angiogenesis and the formation of vascular permeability can lead to fluid leakage in blood vessels, and eventually lead to loss of vision [19]. Anti-VEGF drugs such as ranibizumab and bevacizumab have been widely used in clinics [20, 21]. In addition, they have been shown to be effective in slowing the progression of CNV, however, heterogeneity was observed among patients in invalid samples and who have shorter treatment time [22]. It is assumed that genetic factors may be involved in this period of heterogeneous response, such as variants of complement factor F (CHF), VEGFA, ARMS2 and high-temperature requirement factor A1 (HTRA1) genes [23-26].
HTRA1 encodes a member of the serine proteases trypsin family and regulates the use of insulin-like growth factor (IGF) by cleaving IGF-binding protein and transforming growth factor-β (TGF-β), which is considered regulators of cell growth, angiogenesis and extracellular matrix deposition. Furthermore, the inhibition of TGF-β may result in the overexpression of HTRA1 gene in wet AMD [27] (https://www.ncbi.nlm.nih.gov/gene/5654).

One of common polymorphisms in HTRA1 gene is rs11200638 (wide allele G to mutation allele A) [28]. Dewan et al. reported that the risk allele of the HTRA1 gene (A-allele) can affect the overexpression of its protein, which may then affect the integrity of Bruch’s membrane and stimulate the progression of the CNV stage [29].

In view of the above, we are aware of the critical role of HTRA1 gene and its common rs11200638 polymorphism, and we conducted a comprehensive summary using meta-analysis methods, including 28 different publications (33 case-control studies) [26, 30-57].

**Methods**

**Search Strategy**

We searched relative studies from PubMed and Wanfang databases before 27th Jan, 2020. The keywords were “age-related macular degeneration,” “AMD,” “polymorphism or variant,” and “HTRA1 or high-temperature requirement factor A1.” With these terms, a total of 35 different articles were included from above databases based on our inclusion criteria. Stages of AMD were assigned based on the classification of the Age-Related Eye Disease Study (AREDS) [58].

**Inclusion and Exclusion Criteria**

Included studies were according with (i) the correlation between AMD risk and HTRA1 gene rs11200638 polymorphism; (ii) case-control studies, and (iii) sufficient numbers of each genotypes (AA, AG, and GG) in case and control groups. Studies were excluded if they (i) included no control information; (ii) didn’t contain genotype frequency data, and (iii) were duplicated studies with some other papers [59].

**Data Extraction**

Two authors (Ying Liu and Dong Wei) independently screened all papers that according with the selection criteria. These data included the first author’s last name, publication year, country of origin, ethnicity, Hardy-Weinberg equilibrium (HWE) of control group, genotyping method and AMD disease types (dry and wet AMD). Ethnicity was categorized as Caucasian or Asian. The control subgroups were classified to population-based (PB) and hospital-based (HB) [59].

**Statistical Analysis**

Based on the genotype frequencies for cases and controls, odds ratios (OR) with 95% confidence intervals (CI) were used to measure the strengths of associations[59, 60]. The statistical significance of the OR was determined with the Z test [61]. The heterogeneity assumption among studies was evaluated using a χ^{2}-square-based Q test. If P-value > 0.10 for the Q test was indicated, a lack of heterogeneity among studies, other words, Mantel-Haenszel (fixed-effects model) was chosen, otherwise, the DerSimonian-Laird (random-effects model) was applied [62, 63]. We investigated the correlation between rs11200638 polymorphism and AMD risk by testing whole five genetic models: A versus G, AG versus GG, AA + AG versus GG, AA versus GG and AA versus AG+GG. A sensitivity analysis was performed by omitting studies, one after another, to assess the stability of results. The departure of frequencies of the rs11200638 polymorphism from expectation under HWE was assessed by the Pearson’s χ^{2} test, P < 0.05 was considered significant [64]. The funnel plot was evaluated by Begg’s test, and the publication bias was evaluated by Egger’s test, whose P-value < 0.05 was considered significant [65]. All statistical tests for this meta-analysis were performed using version 10.0 Stata software (StataCorp LP, College Station, TX, USA)[60]. The power and sample size analysis of our meta-analysis was calculated by a program called PS: Power and Sample Size.
Network of gene-interaction of HTRA1 gene

The network of gene-gene interactions for HTRA1 gene was utilized through String online server (http://string-db.org/) to more complete understanding of the role of HTRA1 in AMD [67].

Study searching and their basic information

Using various combinations of key terms, a total of 262 article titles were garnered by a document search using the PubMed (222 titles) and Wanfang (40 titles) databases. As shown in Figure 1, 178 articles were excluded after screening the Abstract sections of the manuscripts. The full texts were then evaluated, and 49 additional articles were excluded due to duplication (7), meta-analysis or systematic analysis (26), clinical trial (10), randomized controlled trial (6). Finally, 35 different articles [26, 30-57, 68-72] were included in our meta-analysis, including 38 case-control studies about HTRA1 gene rs11200638 polymorphism and AMD risk (Table 1) and 27 case-control studies about HTRA1 gene rs11200638 polymorphism and wet or dry AMD risk (Table 2). Five case-control studies [68-72] were not consistent with HWE in control groups. To make our analysis more strict, we deleted above five studies, so there were about 33 case-control studies (8101 cases and 7215 controls) for the whole AMD [26, 30-57], and 22 case-control studies for wet or dry (3938 cases and 4427 controls) studies [26, 30, 31, 33, 34, 40, 41, 43, 44, 46-48, 51, 53-56]. The frequency of the A allele from case group was found to be higher in control individuals (54.2% vs. 36.5%) (Figure 2, Supplementary Table 1). There were 19 case-control studies of Asian population, and 14 from Caucasian population; source of control in 22 case-control studies were from HB, and 11 were from PB; 17 case-control studies were about wet AMD disease, and 5 were about dry disease. Finally, we checked the Minor Allele Frequency (MAF) reported for the five main worldwide populations in the 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/snp/rs11200638): Global (0.290); East Asian (EAS=0.411); European (EUR=0.194); African (AFR=0.257); American (AMR=0.250); and South Asian (SAS=0.340) (Figure 3, Supplementary Table 1). The genotyping methods included polymerase chain reaction-restrictive fragment length polymorphism and matrix-assisted laser desorption/ionization time-of-flight, sequencing, real-time PCR, and TaqMan.

Quantitative Synthesis

Total analysis

Results of the overall meta-analysis were suggestive of increasing associations between this polymorphism and AMD susceptibility in all five genetic models (for example: AA vs. GG: OR = 5.45, 95CI% = 4.26-6.98, \(P < 0.001\)) (Table 3). In order to make this study more convincing and reliable, we detected five studies, which were not according with HWE, finally, we tested the 33 case-control studies. Also significantly increasing correlations were observed in whole genetic models (for example: A-allele vs. G-allele: OR = 2.56, 95%CI = 2.34-2.80, \(P < 0.001\); AA+AG vs. GG: OR = 2.80, 95%CI = 2.49-3.15, \(P < 0.001\)) (Figure 4) (Table 3).

Subgroup analysis

Coming up, we all know that the frequency of A-allele in different races was not the same, so we tried to analysis the relationships by ethnicity subgroups in further, which indicated an incremental statistically association between this polymorphism and both in Asians (A-allele vs. G-allele: OR = 2.51, 95% CI = 2.22-2.83, \(P_{\text{heterogeneity}} < 0.001, P < 0.001\), Figure 4; AA vs. AG+GG: OR = 3.70, 95% CI = 3.16-4.35, \(P_{\text{heterogeneity}} = 0.009, P < 0.001\)) and Caucasian populations (dominant model, OR = 2.94, 95% CI = 2.51-3.45, \(P_{\text{heterogeneity}} < 0.001;\) heterozygote comparison, OR = 2.37, 95% CI = 2.15-2.61, \(P_{\text{heterogeneity}} < 0.001;\) allelic comparison, OR = 2.63, 95% CI = 2.29-3.02, \(P_{\text{heterogeneity}} < 0.001, P < 0.001\), Figure 4) (Table 3). In addition, regular analysis by source of control, also significantly increased risks were detected for this SNP both in PB and HB studies (AG vs. GG: OR = 1.86, 95% CI = 1.58-2.19, \(P_{\text{heterogeneity}} = 0.025, P < 0.001\) for HB; AG vs. GG: OR = 2.16, 95% CI = 1.84-2.53,
$P_{\text{heterogeneity}} = 0.021$, $P < 0.001$ for PB) (Table 3) (Figure 5). AMD have different types and stages, the different of clinical presentation for dry and wet AMD is completely different, so we firmly believed that the correlations existed should be evaluated separately, significant positive associations were found both for dry (such as AA+AG vs. GG: OR = 2.73, 95% CI = 2.13-3.51, $P_{\text{heterogeneity}} = 0.498$, $P < 0.001$, Figure 6A) and wet AMD (for example in AA+AG vs. GG: OR = 3.40, 95% CI = 2.90-3.99, $P_{\text{heterogeneity}} = 0.073$, $P < 0.001$, Figure 6B). Finally, different genotype methods were applied in included studies, we tried to in each method, whether associations may exist in our analysis, we found some positive results in some methods (such as in AA vs. GG model: OR = 7.52, 95% CI = 2.05-27.68, $P_{\text{heterogeneity}} < 0.001$, $P = 0.002$ about TaqMan; OR = 4.30, 95% CI = 2.51-7.35, $P_{\text{heterogeneity}} = 0.073$ about PCR-RFLP, OR = 3.84, 95% CI = 1.53-9.63, $P_{\text{heterogeneity}} = 0.044$ about MassARRAY MALDI-TOF, Figure 7A; OR = 7.00, 95% CI = 5.84-8.39, $P_{\text{heterogeneity}} = 0.677$ about sequencing, OR = 9.83, 95% CI = 5.18-18.65, $P_{\text{heterogeneity}} = 0.817$ about sequencing RT-PCR, Figure 7B) (Table 3).

Bias Diagnosis for publication and sensitivity Analysis

The publication bias was evaluated by both Begg’s funnel plot and Egger’s test. At beginning, the shape of the funnel plots seemed asymmetrical in allele comparison for rs11200638 by Begg’s test, suggesting no publication bias was existed. Then, Egger’s test was applied to provide statistical evidence of funnel plot symmetry. As a result, no obvious evidence of publication bias was observed (A-allele vs. G-allele, $t = 0.89$, $P = 0.38$ for Egger’s test; $z = 0.85$, $P = 0.396$ for Begg’s test, Supplementary Figure 1A,B)(Table 4).

To delete studies which may influence the power and stability of whole study, we applied the sensitive analysis, finally, no sensitive case-control studies were found (Supplementary Figure 2).

Gene-gene network diagram and interaction of online website

String online server indicated that HTRA1 gene interacts with numerous genes. The network of gene-gene interaction has been illustrated in Figure 8.

Discussion

Due to the severe consequences of vision loss caused by AMD, especially advanced AMD (atrophic/dry or neovascular/wet), it is necessary to study its etiology and mechanism, and then develop early diagnostic methods and effective treatments. Today, VEGF inhibitors have been widely regarded as effective drugs in clinical application for CNV (wet AMD) [3, 73, 74]. Therefore, identifying some novel detection markers and target drugs for some different types of AMD is the focus of current and future research. In the introduction, we clarified that genetic factors may help us to search for AMD in potential high-risk groups, which can be prevented and treated in advance.

In our analysis, we selected the HTRA1 gene that can regulate certain growth factors. The rs11200638 polymorphism in HTRA1 is the most common single nucleotide polymorphism (SNP) and has been received attention. However, Kanda et al. [35] demonstrated that there was no HTRA1 gene involved in AMD related SNPs, and its rs11200638 polymorphism did not appear to have an effect on the transcripts. Instead, they found a putative mitochondrial protein (LOC105378525) that may be expressed in the retina in the negative strand, which may be a candidate gene. In fact, they showed that rs11200638 and rs10490924 are in a strong linkage disequilibrium, which is a predicted non-synonymous A69S change in a protein named LOC105378525(LOC387715)/ARMS2. According to their research, rs10490924 was a strong candidate SNP associated with AMD risk, not rs11200638. In addition, Bonyadi et al. [75] conducted a meta-analysis of rs10490924 and found that the combined cigarette smoking and rs10490924 polymorphism may have significant association with AMD risk. We believed rs10490924 was a valuable SNP for AMD, nevertheless, conclusions based on a single study may not be negated by the potential functions for HTRA1 and its SNPs, which need more evidences and support from published and future researches.

Mori et al. first investigated the association between HTRA1 gene rs11200638 polymorphism and risk of AMD
Other more following researchers duplicated their work in different populations and different types of AMD. However, results were confounding, even within same populations, though two published meta-analysis. As we all know, meta-analysis provides a method that can effectively increase the size of the sample by combining data from various related studies, thereby enhancing the statistical power of the analysis to estimate genetic effects [76], which used this method to demonstrate statistically significant genetic associations.

Two previous meta-analysis [77, 78] about rs11200638 polymorphism and AMD have been reported, however, each study has its limitations. For example, Tang et al. just included fourteen case-control studies, two studies [68, 70] were not consistent with HWE, and Tuo et al. actually reported four-source case-control studies, which shouldn’t be combined together [78]. Chen et al. also performed a meta-analysis in the same year including 14 case-control studies, similar limitations were existed [77]. After year of 2008, newly added studies have been published, and to perfect the above deficiencies, we performed an updated meta-analysis to come to a more convincing conclusion about HTRA1 gene rs11200638 polymorphism and AMD susceptibility.

To the best of our knowledge, this is the comprehensive and systematic meta-analysis exploring the associations between HTRA1 gene rs11200638 polymorphism and AMD risk; it involved about 8101 AMD individuals and 7215 controls. Increased associations were found in the whole group, in Asian and Caucasian subgroups, source of control subgroup, and dry/wet sub-types of AMD, different genotyping methods (Sequencing, TaqMan, PCR-RFLP, RT-PCR and MassARRAY MALDI-TOF), which means that A-allele or AA genotype is the risk factor for AMD, in other words, if individuals carry on this SNP from peripheral blood test, which may indicate that it is possible to increase the occurrence of AMD for them in present time or at some point in the future. Therefore, this polymorphism may be helpful in screening vulnerable populations for AMD in advance. In addition, the power of present study was 1.00, which suggested our conclusions were stable and convincing.

In addition, the online analysis system-String was applied to predict potential and functional partners related to HTRA1, which can help us to better understand the value for detection and concern. Finally, ten genes were predicted. Among them, the highest score of association was ACAN (0.943), however, so far, no research has been reported between this gene and AMD and interaction between this gene and HTRA1. Future research should be payed attention to above information, which may be in favor of AMD early detection/prevention and intervention. In other partners, ARMS2 and CFH have been shown to associate with AMD. The ARMS2 and HTRA1 genes are located nearby on the 10q26 chromosome in a strong linkage disequilibrium. Significant association was observed that ARMS2 rs10490924 was related response to ranibizumab treatment among wet AMD subjects [71]. CFH gene T1277C polymorphism is strong associated with both wet and dry AMD and may contribute to the inflammation in the pathogenesis of AMD [79]. As for the rest interaction genes (CLPP, CTRC, YME1L1, HSPD1, RPL34, CLPX and PLEKHG4) both had moderate score and no literature to support. It seems that above ten genes associated with HTRA1 came from text mining scores, which were derived from the co-occurrence of gene/protein names in related abstracts. In addition, it was important considered the occurrence of the LOC105378525 (LOC387715) and its polymorphism (A69S, rs10490924) as the main factor for AMD reported by Kanda et al (2007) [35], which should be added in the network of HTRA1 related genes. In a word, we should deep explore these partners of HTRA1 gene, and gene-gene interactions in the development of AMD in the next step.

There are some inherent limitations of our study should be declared. First, further studies should focus on Mixed and African populations, which was vacant in present analysis. Second, gene-gene and gene-environment interactions were not well analyzed. It is possible that specific environmental and lifestyle factors alter the associations between HTRA1 rs11200638 polymorphism and AMD, including age, diabetes, smoking, familial history, and hypertension. Third, vision is the most concerned-clinical indicator of AMD, future studies should include the value of the vision and analyze the relationships between rs11200638 polymorphism and the degree of visual impairment, which may help us to better detect disease progression.

In conclusion, our present meta-analysis suggests that HTRA1 rs11200638 polymorphism may be a risk factor.
for the susceptibility of AMD, larger and more comprehensive studies should be performed in the future.

**Abbreviations**

AMD: age-related macular degeneration; GWAS: genome-wide association studies; HTRA1: high-temperature requirement factor A1; CNV: choroidal neovascularization; VEGF: vascular endothelial growth factor; AREDS: Age-Related Eye Disease Study; SNP: single nucleotide polymorphism; HB: hospital-based; PB: population-based; SOC: source of control; PCR-RFLP: polymerase chain reaction followed by restriction fragment length polymorphism; MALDI-TOF: a chip-based matrix-assisted laser-desorption/ionization time-of-flight.

**Declarations**

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Not applicable.

**Author contributions**

YL and HJ designed the study and drafted the manuscript; DW extracted, analyzed, interpreted the data, and collected the clinical data; DW and WL performed the targeted sequencing, analyzed and interpreted the data; DW and WL participated in the study coordination and revised the manuscript. All authors read and approved the final version of the manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its supplementary information files.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1Ophthalmic function room, Hongqi Hospital Affiliated to Mudanjiang Medical College, Mudanjiang, 157000, Heilongjiang Province, China. 2Department of Ophthalmology (three disease areas), Hongqi Hospital Affiliated to Mudanjiang Medical College, Mudanjiang, 157000Heilongjiang Province, China. 3Department of Critical Medicine, Second People's Hospital of Mudanjiang, 157000Heilongjiang Province, China
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### Tables

Table 1 Characteristics of included studies in HTRA1 rs11200638 polymorphism and AMD risk.

| Author   | Year | Country   | Ethnicity | type   | Case | Control |
|----------|------|-----------|-----------|--------|------|---------|
| Tian     | 2012 | China     | Asian     | AMD    | 532  | 468     |
| Ruamviboonsuk | 2017 | Thailand  | Asian     | wet    | 377  | 1073    |
| Chu      | 2008 | China     | Asian     | wet    | 144  | 126     |
| Losonczy | 2011 | Hungary   | Caucasian | AMD    | 103  | 95      |
| Tuo      | 2008 | USA       | Caucasian | AMD    | 142  | 132     |
| Tuo      | 2008 | USA       | Caucasian | AMD    | 330  | 191     |
| Tuo      | 2008 | USA       | Caucasian | AMD    | 272  | 555     |
| Tuo      | 2008 | USA       | Caucasian | AMD    | 46   | 22      |
| Chan     | 2007 | USA       | Caucasian | AMD    | 52   | 13      |
| Kanda    | 2007 | USA       | Caucasian | AMD    | 457  | 280     |
| Name       | Year | Country        | Ethnicity | Disease | Gender | Age M | Age F |
|------------|------|----------------|-----------|---------|--------|-------|-------|
| Cheng      | 2013 | China          | Asian     | wet     | 93     | 93    |
| Liang      | 2012 | China          | Asian     | wet     | 161    | 150   |
| Jiang      | 2008 | China          | Asian     | wet     | 159    | 140   |
| Lee        | 2010 | Korea          | Asian     | wet     | 137    | 187   |
| Lin        | 2008 | China-Taiwan   | Asian     | AMD     | 95     | 90    |
| Kaur       | 2008 | India          | Asian     | AMD     | 229    | 184   |
| Xu         | 2007 | China          | Asian     | wet     | 121    | 132   |
| Tam        | 2008 | China-Hong Kong| Asian     | wet     | 163    | 183   |
| Mori       | 2007 | Japan          | Asian     | AMD     | 123    | 133   |
| Askari     | 2015 | Iran           | Asian     | AMD     | 120    | 120   |
| Lana       | 2018 | Brazil         | Caucasian | AMD     | 204    | 166   |
| Kaur       | 2013 | India          | Asian     | AMD     | 198    | 145   |
| Ng         | 2016 | Hong Kong      | Asian     | wet     | 194    | 183   |
| Kaur       | 2013 | India          | Caucasian | AMD     | 616    | 426   |
| Chen       | 2013 | China          | Asian     | AMD     | 158    | 157   |
| Kondo      | 2007 | Japan          | Asian     | AMD     | 73     | 94    |
| Leveziel   | 2007 | France         | Caucasian | wet     | 118    | 116   |
| Weger      | 2007 | Austria        | Caucasian | wet     | 242    | 157   |
| Lu         | 2007 | China          | Asian     | wet     | 90     | 106   |
| Cruz-González | 2013 | Spain       | Caucasian | AMD     | 121    | 91    |
| Mohamad    | 2019 | Malaysia       | Asian     | wet     | 145    | 145   |
| Yang       | 2010 | China          | Asian     | wet     | 109    | 150   |
| Chen       | 2008 | USA            | Caucasian | AMD     | 776    | 294   |
| Zeng       | 2011 | China          | Caucasian | AMD     | 1335   | 509   |
| Li         | 2015 | China          | Asian     | AMD     | 146    | 145   |
| Yang       | 2018 | China          | Asian     | AMD     | 201    | 201   |
Table 2 Characteristics of included studies in HTRA1 rs11200638 polymorphism and wet/dry AMD risk, respectively.

| Author          | Year | Country                | Ethnicity | type | Case | Control | SOC         |
|-----------------|------|------------------------|-----------|------|------|---------|-------------|
| Lin             | 2008 | China-Taiwan           | Asian     | dry  | 52   | 90      | HB          |
| Chan            | 2007 | USA                    | Caucasian | dry  | 18   | 13      | HB          |
| Mori            | 2007 | Japan                  | Asian     | dry  | 19   | 116     | HB          |
| Askari          | 2015 | Iran                   | Asian     | dry  | 32   | 120     | HB          |
| Ruamviboonsuk   | 2017 | Thailand               | Asian     | wet  | 377  | 1073    | PB          |
| Cheng           | 2013 | China                  | Asian     | wet  | 93   | 93      | HB          |
| Ng              | 2016 | Hong Kong              | Asian     | wet  | 194  | 183     | PB          |
| Liang           | 2012 | China                  | Asian     | wet  | 161  | 150     | HB          |
| Chu             | 2008 | China                  | Asian     | wet  | 144  | 126     | HB          |
| Jiang           | 2008 | China                  | Asian     | wet  | 159  | 140     | HB          |
| Lee             | 2010 | Korea                  | Asian     | wet  | 137  | 187     | HB          |
| Lin             | 2008 | China-Taiwan           | Asian     | wet  | 43   | 90      | HB          |
| Xu              | 2007 | China                  | Asian     | wet  | 121  | 132     | HB          |
| Tam             | 2008 | China-Hong Kong        | Asian     | wet  | 163  | 183     | HB          |
| Chan            | 2007 | USA                    | Caucasian | wet  | 31   | 13      | HB          |
| Leveziel        | 2007 | France                 | Caucasian | wet  | 118  | 116     | HB          |
| Mori            | 2007 | Japan                  | Asian     | wet  | 104  | 116     | HB          |
| Name     | Year  | Region     | Ethnicity | Type  | Cases | Controls | Study Type |
|----------|-------|------------|-----------|-------|-------|----------|------------|
| Askari   | 2015  | Iran       | Asian     | wet   | 88    | 120      | HB         |
| Weger    | 2007  | Austria    | Caucasian | wet   | 242   | 157      | PB         |
| Lu       | 2007  | China      | Asian     | wet   | 90    | 106      | HB         |
| Mohamad  | 2019  | Malaysia   | Asian     | wet   | 145   | 145      | HB         |
| Yang     | 2010  | China      | Asian     | wet   | 109   | 150      | HB         |
| Chen     | 2008  | USA        | Caucasian | wet   | 470   | 294      | HB         |
| Chen     | 2008  | USA        | Caucasian | dry   | 306   | 294      | HB         |
| Zeng     | 2011  | China      | Caucasian | dry   | 341   | 509      | PB         |
| Zeng     | 2011  | China      | Caucasian | wet   | 994   | 509      | PB         |
| Matuskova| 2020  | Czech Republic | Caucasian | wet   | 307   | 191      | HB         |

Table 3 Results of the meta-analysis on HTRA1 rs11200638 polymorphism and AMD risk in total and types of subgroups.
| Variables      | N   | Case/Control | A-allele vs. G-allele | AG vs. GG | AA+AG |
|---------------|-----|--------------|-----------------------|----------|-------|
|               |     |              | OR(95%CI) | P   | P     | OR(95%CI) | P      | P     | OR(95%CI) | P   | P     |
| Total         | 38  | 8582/7452    | 2.39(2.12-2.69) | 0.000 | 0.000 | 1.91(1.69-2.16) | 0.000 | 0.000 | 2.63(2 | 0.000 | 0.000 |
| HWE           | 33  | 8101/7215    | 2.56(2.34-2.80) | 0.000 | 0.000 | 1.98(1.76-2.23) | 0.001 | 0.000 | 2.80(2 | 0.000 | 0.000 |
| Ethnicity     |     |              |          |     |       |          |       |       |       |     |       |
| Asian         | 19  | 3424/4004    | 2.51(2.22-2.83) | 0.000 | 0.000 | 1.67(1.47-1.88) | 0.167 | 0.000 | 2.68(2 | 0.000 | 0.000 |
| Caucasian     | 14  | 4677/3211    | 2.63(2.29-3.02) | 0.000 | 0.000 | 2.37(2.15-2.61) | 0.140 | 0.000 | 2.94(2 | 0.000 | 0.000 |
| SOC           |     |              |          |     |       |          |       |       |       |     |       |
| HB            | 22  | 3589/3273    | 2.56(2.28-2.88) | 0.000 | 0.000 | 1.86(1.58-2.19) | 0.027 | 0.000 | 2.81(2 | 0.000 | 0.000 |
| PB            | 11  | 4512/33942   | 2.55(2.18-2.99) | 0.000 | 0.000 | 2.16(1.84-2.53) | 0.021 | 0.000 | 2.80(2 | 0.000 | 0.000 |
| AMD type      |     |              |          |     |       |          |       |       |       |     |       |
| Wet           | 17  | 3476/3579    | 3.03(2.59-3.55) | 0.000 | 0.000 | 2.11(1.81-2.46) | 0.038 | 0.000 | 3.40(2 | 0.000 | 0.000 |
| Dry           | 5   | 462/848      | 2.36(1.71-3.24) | 0.750 | 0.000 | 1.33(0.76-2.32) | 0.316 | 0.316 | 2.08(1 | 0.000 | 0.000 |
| Genotyping    |     |              |          |     |       |          |       |       |       |     |       |
| Others        | 5   | 3004/2599    | 2.55(2.22-2.94) | 0.027 | 0.000 | 2.14(1.67-2.73) | 0.006 | 0.000 | 2.85(2 | 0.000 | 0.000 |
| Sequencing    | 14  | 2613/2332    | 2.84(2.61-3.09) | 0.237 | 0.000 | 2.08(1.81-2.41) | 0.252 | 0.000 | 3.19(2 | 0.000 | 0.000 |
| TaqMan        | 4   | 591/524      | 2.66(1.43-4.94) | 0.000 | 0.000 | 2.79(1.31-5.91) | 0.008 | 0.008 | 3.61(1 | 0.000 | 0.000 |
| PCR-RFLP      | 6   | 1037/1121    | 1.98(1.72-2.26) | 0.105 | 0.000 | 1.76(1.45-2.14) | 0.611 | 0.000 | 2.08(1 | 0.000 | 0.000 |
| RT-PCR        | 2   | 509/293      | 2.91(2.30-3.69) | 0.755 | 0.000 | 2.08(1.51-2.86) | 0.643 | 0.000 | 2.90(2 | 0.000 | 0.000 |
| MassARRAY     | 2   | 347/346      | 2.18(1.39-3.49) | 0.043 | 0.001 | 1.46(0.95-2.24) | 0.213 | 0.088 | 2.22(2 | 0.000 | 0.000 |

$P_h$: value of $Q$-test for heterogeneity test; $P$: Z-test for the statistical significance of the OR
Table 4 Publication bias tests (Begg’s funnel plot and Egger’s test for publication bias test) for HTRA1 rs11200638 polymorphism.

| Genetic type          | Coefficient | Standard error | t   | P value | 95%CI of the intercept |
|-----------------------|-------------|----------------|-----|---------|------------------------|
| A-allele vs. G-allele | 0.211       | 0.924          | 0.23| 0.820   | (-1.673-2.096)         |
| AG vs. GG             | -0.031      | 0.500          | -0.06| 0.951   | (-1.051-0.989)         |
| AA+AG vs. GG          | -0.045      | 0.532          | -0.08| 0.933   | (-1.130-1.040)         |
| AA vs. GG             | 0.297       | 0.382          | 0.78| 0.441   | (-0.481-1.076)         |
| AA vs. AG+GG          | 0.365       | 0.438          | 0.83| 0.412   | (-0.529-1.258)         |

Supplementary Materials

Supplementary Table 1. **Supplementary Table 1** Allele Frequency from 1000 Genomes Browser and present study.

Supplementary Figure 1. A: Begg’s funnel plot for publication bias test (A-allele vs. G-allele). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size. B: Egger’s publication bias plot (A-allele vs. G-allele).

Supplementary Figure 2. Sensitivity analysis between HTRA1 gene rs11200638 polymorphism and AMD risk (A-allele vs. G-allele).
35 different articles about betweeen HTRA1 gene rs11200638 were excluded after reading full article evaluation.
Figure 1

Flowchart illustrating the search strategy used to identify association studies for HTRA1 gene rs11200638 polymorphism and AMD risk.
Figure 2

The MAF of minor-allele (mutant-allele) for HTRA1 gene rs11200638 polymorphism from the 1000 Genomes online database and present analysis. EAS: East Asian; EUR: European; AFR: African; AMR: American; SAS: South Asian.
A-allele frequencies for the HTRA1 gene rs11200638 polymorphism among cases/controls stratified by ethnicity. Vertical line, allele frequency; Horizontal line, case/control groups.

| Study ID | Asian |
|----------|-------|
|          | Tian (2012) |
|          | Ruamviboonsuk (2017) |
|          | Cheng (2013) |
|          | Liang (2012) |
|          | Jiang (2008) |
|          | Lee (2010) |
|          | Lin (2008) |
|          | Kaur (2008) |
|          | Xu (2007) |
|          | Tam (2008) |
|          | Mori (2007) |
|          | Askari (2015) |
|          | Kaur (2013) |
|          | Ng (2016) |
|          | Chen (2013) |
|          | Kondo (2007) |
|          | Li (2015) |
|          | Chu (2008) |
|          | Yang (2018) |
Figure 4

Forest plot of AMD risk associated with HTRA1 gene rs11200638 polymorphism (A-allele vs. G-allele) by ethnicity subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.
Study ID

HB
Tian (2012)
Losonczy (2011)
Chan (2007)
Kanda (2007)
Cheng (2013)
Liang (2012)
Jiang (2008)
Lee (2010)
Lin (2008)
Kaur (2008)
Xu (2007)
Tam (2008)
Mori (2007)
Askari (2015)
Lana (2018)
Chen (2013)
Kondo (2007)
Leveziel (2007)
Li (2015)
Chu (2008)
Yang (2018)
Matušková (2020)
Subtotal (I-squared = 40.8%, p = 0.025)

PR
Figure 5

Forest plot of AMD risk associated with HTRA1 gene rs11200638 polymorphism (AG vs. GG) by source of control subgroup.
Figure 6

Forest plot of AMD risk associated with HTRA1 gene rs11200638 polymorphism (AA+AG vs. GG) by AMD type subgroup. A: wet AMD; B: dry AMD.
Figure 7
Forest plot of AMD risk associated with HTRA1 gene rs11200638 polymorphism (AA vs. GG) by genotyping methods subgroup. A: random model; B: fixed model.

Figure 8
Human HTRA1 interactions network with other genes obtained from String server. At least 10 genes have been...
indicated to correlate with HTRA1 gene. ACAN: aggrecan core protein; ARMS2: age-related maculopathy susceptibility; CLPP: ATP-dependent Clp protease proteolytic subunit; CTRC: chymotrypsin-C; YME1L1: ATP-dependent zine metalloprotease YME1L1; CFH: complement factor H; HSPD1: 60 kDa heat shock protein; RPL34: 60S ribosomal protein L34; CLPX: ATP-dependent Clp protease ATP-binding subunit clpX-like; PLEKHG4: Puratrophin-1.

**Supplementary Files**

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