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# Role of *ARMS2/HTRA1* risk alleles in the pathogenesis of neovascular age-related macular degeneration

**Yang Pan, Takeshi Iwata***

**Abstract:**

Age-related macular degeneration (AMD) is one of the leading causes of severe irreversible blindness worldwide in the elderly population. AMD is a multifactorial disease mainly caused by advanced age, environmental factors, and genetic variations. Genome-wide association studies (GWAS) have strongly supported the link between *ARMS2/HTRA1* locus on chromosome 10q26 and AMD development, encompassing multiple variants, rs10490924 (c.205G > T, p.A69S in *ARMS2*), insertion/deletion (del443/ins54 in *ARMS2*), and rs11200638 (in *HTRA1* promoter region). In this comprehensive review, we provide an overview of the role played by *ARMS2/HTRA1* risk alleles in neovascular AMD pathogenesis, covering GWAS, *in vitro* studies, and animal models, shedding light on their underlying molecular genetic mechanisms. Further extensive research is also imperative, including confirmation of these findings, identifying novel treatment targets, and advancing primary and secondary prevention strategies for AMD.

**Keywords:**

Age-related macular degeneration, *ARMS2*, gene expression, genome-wide association studies, *HTRA1*

## Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among individuals aged 55 years and older in the developed world.[1] It accounts for 6%–9% of legal blindness cases worldwide and is projected to affect 288 million individuals by 2024.[2] The clinical classification systems for early-stage AMD typically categorize it based on drusen size, with medium-size drusen classified as early AMD and large drusen as intermediate AMD. The risk of progressing to late-stage AMD, such as neovascular AMD (nAMD) and/or any geographic atrophy (GA), is highest when large drusen are present along with pigmentary changes. GA, also known as dry AMD, is characterized by confluent atrophy of photoreceptors, retinal pigment epithelial (RPE), and choriocapillaris, leading to scotoma.[3] In AMD at any stage, new vessels may invade the outer retina, subretinal space, or sub-RPE space, giving rise to macular neovascularization (MNV), the hallmark lesion of nAMD.[4] The exudative stage of nAMD (also called exudative or wet AMD) becomes evident when these new vessels leak or rupture, causing fluid accumulation and/or hemorrhages and vision distortion and deterioration. Without treatment, exudative MNV typically results in extensive fibrosis with severe central vision loss.

In 2014, a meta-analysis of pooled global population-based studies involving individuals aged 45 to 85 years estimated the prevalence of AMD as 8.69%.[2] Notably, this prevalence varies among racial and ethnic groups, with 12.3% in people of European descent, 10.4% in those of Latin American heritage, 7.5% in individuals of African descent, and 7.4% in people of Asian ancestry.

*NHO Tokyo Medical Center, National Institute of Sensory Organs, Tokyo, Japan*

***Address for correspondence:**
Dr. Takeshi Iwata, 2-5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902, Japan.
E-mail: takeshi.iwata@kankakuki.jp

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These differences are predicted to arise from environmental or genetic factors. A reanalysis of the Multi-Ethnic Study of Atherosclerosis data, considering common factors such as smoking, body mass index, inflammatory factors, diabetes, and alcohol, failed to explain these variations.[5]

To gain a better genetic understanding across populations, Fritsche *et al*. compared the minor allele frequency (MAF) of the known AMD risk variants among ancestry groups in the 1000 Genomes Project.[6] They found significantly higher MAFs for the *ARMS2/HTRA1* locus in Asian populations (40.2%) compared to Western populations (19.9%), although the *CFH* variant rs10737680 showed similar MAFs (European: 59.1% and East Asian: 57.9%).[6] In addition, in people of European descent, the ratio of nAMD to GA is approximately 1:1 across all age groups. In contrast, a meta-analysis of data from ten different Asian countries found a 3:1 ratio of nAMD to GA.[7] These findings suggest that the *ARMS2/HTRA1* risk variant may predispose individuals toward nAMD, whereas CFH variants might be stronger risk factors for GA. However, the underlying biological mechanism in Asian populations remains elusive, despite the greater significance of the genetic risk in *ARMS2/HTRA1* locus. Understanding the molecular mechanism of this genetic risk is crucial for elucidating the etiology of AMD. In this review, we explore the literature on several key aspects: (1) the role of the *ARMS2/HTRA1* locus in Asian populations, (2) animal models related to ARMS2, (3) animal models related to *HTRA1*, (4) enhancers binding to *ARMS2* and its effect on *HTRA1* transcription, and (5) *HTRA1* expression in human samples.

## Genetic Dissection of *ARMS2/HTRA1*

The initial evidence of genetic involvement in AMD susceptibility came from familial aggregation and twin studies.[8] The heritability of late AMD is estimated to be as high as 71%, greater than that of most complex age-related diseases.[1] In 2005, several independent genome-wide association studies (GWAS) identified two major loci associated with an increased risk of AMD in White populations: *CFH* (rs1061170, p.Y402H) on chromosome 1q31.3[9-12] and *ARMS2/HTRA1* on chromosome 10q26.[13,14] However, ethnic differences have been observed in the prevalence of nAMD and GA.[15] In Asian populations, nAMD is significantly more common than GA,[7] which differs from White populations where the prevalence is relatively equal.[15]

To gain a better genetic understanding of the Asian population, in 2006 and 2007, our group validated the association between the *CFH* loci and nAMD[16] as well as *ARMS2/HTRA1* loci and nAMD[17] in the Japanese population using temperature gradient capillary electrophoresis and direct sequencing, respectively. Interestingly, we found that the frequencies of each haplotype in the *CFH* gene were significantly different from those in published White populations. Moreover, the allelic association test for rs11200638 (in the *HTRA1* promoter region) yielded $P < 10^{-11}$. Single-nucleotide polymorphism (SNP) rs11200638 conferred disease risk in an autosomal recessive fashion, with an odds ratio of 10.1 (95% confidence interval: 4.36, 23.06), significantly higher than that reported in previous papers. In 2009, our group conducted the first GWAS of Japanese patients with nAMD, classified as 5b by Seddon *et al*.,[18] using the Affymetrix GeneChip Human Mapping 500K Array and TaqMan assay.[19] Japanese nAMD patients showed a stronger association with *ARMS2/HTRA1* locus than *CFH* locus, in contrast to individuals with late AMD in White populations. In addition, lower associations of rs1061170 and other variations in the *CFH* gene were reported in Japanese,[20] Chinese,[21-23] and Korean populations,[24] suggesting that, in Asian populations, the *ARMS2/HTRA1* locus plays a more significant role compared to the *CFH* locus.

To date, numerous genetic association studies have been conducted. In the current largest GWAS of AMD, carried out by the International AMD Genomics Consortium, 34 independent loci were identified to be associated with late AMD.[25] These 52 variants have been estimated to explain almost 50% of the variability of AMD in Europeans, with the *ARMS2/HTRA1* locus alone accounting for approximately 30% of this genetic variance. Notably, the most robust genetic signal for AMD, with odds ratios of 2.81, was discovered in the *ARMS2/HTRA1* locus.[25] Consequently, the *ARMS2/HTRA1* locus stands out as one of the most strongly associated loci for AMD, suggesting that either *ARMS2*, *HTRA1*, or both may be the causal genes for AMD risk.

It is worth mentioning that the *ARMS2* and *HTRA1* genes are separated by approximately 4 Kbp adjacent to each other on chromosome 10q26 in the same linkage disequilibrium (LD) block.[26] Although several sophisticated fine-mapping approaches, based on Bayesian models, are currently employed to identify the most likely causal variants, prioritizing a single-credible SNP over highly correlated SNPs with absolute LD remains a challenge.[27] Thus, GWAS has not been able to definitively determine which variant (s) in *ARMS2/HTRA1* locus are responsible for AMD risk. Furthermore, GWAS do not specify whether one or both genes are affected by the causal variant. Consequently, the functional implications of these two genes will be explored in the next section using animal models.

## ARMS2 Animal Models

ARMS2 is a primate-specific gene that encodes an 11.4-kDa secreted protein.[28,29] Its localization and

function have been disputed since its discovery.[28,29] Initially, it was believed to confer AMD risk, potentially through mitochondrial-related pathways.[30] However, later attempts to replicate its mitochondrial localization were unsuccessful.[31] In 2010, Kortvely *et al*. discovered that the endogenous ARMS2 protein is primarily localized to the choroid pillars in human eyes.[32] This localization suggests that it is a component of the extracellular matrix, corresponding to the principal sites of drusen formation. Given the pivotal role of the extracellular matrix in AMD's progression, which often involves abnormal deposition of extracellular debris in the macula, it was hypothesized that ARMS2 protein may be necessary for proper matrix function.

To determine the function of the ARMS2 protein in the choroid and retina, in 2014, our group generated *ARMS2* transgenic (Tg) mice using the CMV early enhancer/chicken beta actin (CAG), resulting in the ubiquitous expression of human ARMS2 protein in mice.[33] Furthermore, numerous studies have consistently demonstrated that a conserved primate-specific missense polymorphism, rs10490924 (p.A69S), affects the *ARMS2* coding sequence and contributes to over 50% of AMD susceptibility.[13] The A69S alteration was suggested to potentially impact its function within the mitochondria or trigger RPE apoptosis through some toxic effects of ARMS2 (A69S).[30] Consequently, we also created *ARMS2* (p.A69S) Tg mice using the same promoter. Nevertheless, histopathological examination and immunohistochemistry analysis of aged *ARMS2* Tg and *ARMS2* (p.A69S) Tg mice revealed no retinal or choroid abnormalities compared to age-matched controls.

Moreover, smoking stands out as the most potent modifiable risk factor for AMD development.[26] Given the intricate nature of AMD pathogenesis, our research group also combined direct inhalation of cigarette smoke with advanced age in these Tg mouse models, aiming to establish a link between the ARMS2 protein and AMD risk.[33] Similarly, none of the aged *ARMS2* and *ARMS2* (p.A69S) Tg mice exposed to mainstream cigarette smoke displayed any abnormal retinal changes or subretinal deposits. In 2015, Liu *et al*. developed an alternative Cre-conditional human *ARMS2* Tg mouse model using the cytomegalovirus (CMV) promoter. Their findings align with our results, supporting the conclusion that ubiquitous overexpression of human ARMS2 protein does not lead to a typical AMD phenotype in aged mice.[34]

Furthermore, a common protective haplotype arises due to a premature stop codon (rs2736911, p.R38X) in the *ARMS2* gene, leading to the termination of ARMS2 protein synthesis. This means that homozygous carriers lack the ARMS2 protein. However, this variant is not associated with AMD.[35] A recently published paper also mentioned that *ARMS2* expression cannot be detected in human ocular samples by RNA sequencing, real-time quantitative polymerase chain reaction (RT-qPCR), or Western blotting.[36] Therefore, the function of *ARMS2* risk variants might not be strictly associated with ARMS2 protein, including its transcription and presumed function.

Notably, *ARMS2* is adjacent to the *HTRA1* gene and is in LD with the *HTRA1* gene.[26] Thus, its function might be closely associated with the *HTRA1* protein rather than the ARMS2 protein. In these three published Tg mouse models, the *ARMS2* transgene was inserted randomly in the mouse genome, and its interactions with *HTRA1* could not be examined. The appropriate method for investigating the function of the *ARMS2* gene in mice could entail creating a knock-in model to introduce either the human ARMS2 gene or its mutant into the corresponding *HtrA1* promoter region.

## *HTRA1* Mouse Models

The serine protease *HTRA1*, among the most extensively studied members within the mammalian HTRA family (*HTRA1*-4), is a 50-kDa secreted protein.[26] This protein consists of an N-terminal signal peptide, followed by an insulin/insulin-like growth factor-binding protein domain, a Kazal inhibitor domain (Kaz), a trypsin-like serine protease domain, and one postsynaptic density protein 95, *Drosophila disc* large tumor suppressor and zonula occludens-1 domain.[37] Convincing evidence has supported the pivotal role of *HTRA1* in several important biological processes and intricate cellular singling pathways, including cleaving extracellular matrix proteins, regulating signaling pathways (transforming growth factor-beta [TGF-β], IGF, and TSP1-CD47), and controlling mammalian target of rapamycin signaling by tuberous sclerosis 2 degradation. Dysregulation of *HTRA1* has been implicated in the pathogenesis of several diseases, including cancer, arthritis, musculoskeletal disorder, cerebral small vessel disease, and AMD.[26,38,39]

### *Htra1* knockout mouse model

In 2005, the first study of mouse *Htra1* knockout (KO) model was published, in which *Htra1* exon 1 was replaced with β-galactosidase cDNA. This study aimed to investigate the role of *HTRA1* in the pathogenesis of arthritis.[40] To assess the impact of *HTRA1* deficiency in the retina, our research group crossed this *Htra1* KO mouse model with C57BL/6J mice line. Subsequently, we conducted fundus imaging, hematoxylin and eosin (H and E) staining, and electroretinogram measurements, including rod and mixed responses and oscillatory potentials. Interestingly, no significant

physiological or histological effects of Htra1 deficiency were observed in the mouse retina at 12 months. These findings were presented at the 2008 Association for Research in Vision and Ophthalmology annual meeting.[41]

Consistent with our results, a study conducted by Jones *et al*. in 2011 also examined *Htra1* KO mice and found that these mice did not exhibit any features of polypoidal choroidal vasculopathy (PCV) or choroidal neovascularization (CNV), as determined by H and E staining, fluorescein angiography, indocyanine green angiography (ICGA), or spectral-domain optical coherence tomography (OCT).[42,43] Furthermore, the following year, Zhang *et al*. observed reduced vascular development and heightened small of mothers against decapentaplegic (SMAD) signaling in the retinas of *Htra1* KO mice, suggesting a critical role for *HTRA1* in regulating angiogenesis through the TGF-β signaling pathway.[43]

### *HTRA1* transgenic models

To further investigate the impact of *HTRA1* overexpression in the retina, we developed *Htra1* Tg mice using the CAG promoter for ubiquitous mouse *Htra1* overexpression.[33] The *Htra1* levels were 3.0-fold higher in the retinas of 12-month-old *Htra1* Tg mice compared to age-matched controls. We observed fluorescein diapedesis in the fundi of *Htra1* Tg mice, indicating the breakdown of the blood–retinal barrier and exacerbation of retinal vascular permeability. In addition, low fluorescence lesions were detected in the choroids of *Htra1* Tg mice through ICGA, suggesting the presence of abnormally branched vessel networks. Furthermore, OCT revealed the presence of radial capillary branching that extended from the choroid, traversing the RPE and reaching into the retina. The formation of radial CNV was further explored through H and E staining and immunostaining with anti-CD-31, an endothelial cell marker. Moreover, *Htra1* Tg mice exhibited ruptures and deficiencies in Bruch's membranes (BMs), as revealed by Elastica van Gieson staining. Notably, 18.2% of *Htra1* Tg mice developed CNV, whereas none of the age-matched WT mice exhibited this condition. Recently, Ahamed *et al*. conducted a more in-depth analysis of this same *Htra1* Tg mouse model and identified signs of subclinical inflammation, which could have led to increased RPE cell activation and angiogenic potential.[44]

Simultaneously, *Htra1* Tg mice were exposed to mainstream cigarette smoke, as described in *ARMS2* Tg mice.[33] Surprisingly, 20% of them developed CNV by the age of 12 months. Although no CNV was observed in the control group exposed to air, equivalent damage was also observed in WT mice exposed to mainstream cigarette smoke. This suggests that exposure to mainstream cigarette smoke triggered CNV, and the formation of CNV was independent of the level of *Htra1* expression. Due to dense deposits in the subretinal space of aged *Htra1* Tg mice, electron microscopy was also performed, revealing abnormal deposits or vacuolization in the subretinal space of *Htra1* Tg mice exposed to mainstream cigarette smoke.[33]

Several other research groups have also studied the effects of overexpressing human *HTRA1* or murine *Htra1* on ocular phenotypes using mouse or zebrafish models. They employed different promoters to explore the association between *HTRA1* overexpression and AMD risk. In 2011, an investigation of human *HTRA1* overexpression in mouse RPE (Tg 73), driven by the CMV-IE enhancer/promoter and VMD2 promoter, revealed key features of PCV in 59% of the 114 *HTRA1* Tg 73 mice, beginning at 3 weeks, and developed occult CNV in 50% of PCV$^{+}$ mice older than 11 months.[42] Simultaneously, another group compared RPE and BM defects in their *Htra1* Tg mouse line, which overexpresses murine *Htra1* specifically in RPE using the Rpe65 promoter.[45] They found altered elastogenesis in the BM through fibulin-5 cleavage. In 2014, human *HTRA1* Tg mice (Tg44), driven solely by the *VMD2* promoter (expressing *HTRA1* in RPE), exhibited a more consistent range of human PCV-like features; 94.7% of Tg 44 mice developed PCV-like features at 4–5 weeks old.[46] To investigate whether the proteolytic activity of *HTRA1* was crucial for PCV development, they generated two other Tg lines (Tg33 and Tg 26) expressing the protease-inactive mutant human *HTRA1* (S328A) in mouse RPE using the human *VMD2* promoter.[47] Neither line exhibited any PCV phenotype. Similarly, intravitreal delivery of an *HTRA1* inhibitor targeting the catalytic site of *HTRA1* effectively prevented PCV initiation.[47] Furthermore, Tg zebrafish that overexpressed human *HTRA1* in rod photoreceptors exhibited photoreceptor death and lipofuscin accumulation, similar to early AMD findings.[48] In summary, all of these studies involving *HTRA1*/*Htra1* animal models suggest that *HTRA1* plays a critical role in AMD pathogenesis.

## The Impact of *ARMS2/HTRA1* Variants on *HTRA1* Transcription

In *ARMS2/HTRA1* locus, seven SNPs have been identified within a 17-kilobase region on chromosome 10q26.[49] These SNPs, including rs10490924, rs3750846, insertion/deletion (del443ins54), rs11200638, rs1049331, and rs2293870, have been widely studied due to their strong associations with the risk of AMD. Notably, the most prominently associated haplotype within

the *ARMS2/HTRA1* locus comprises three variants: rs10490924 (c. 205G > T, resulting in p.A69S in *ARMS2*), the in/del mutation (consisting of a 443-base pair deletion and an adjacent 54-base pair insertion in the 3′-untranslated region of *ARMS2* and its flanking region), and rs11200638 (located within the *HTRA1* promoter region).[26] It is worth highlighting that these three highly associated variants are found within the same LD block. Consequently, despite large-scale GWAS, it has proven challenging to definitively pinpoint which specific variant within this locus is responsible for the observed AMD association. As a result, researchers have turned their attention to elucidating the molecular mechanisms underlying how these genetic variants may influence the regulation of *HTRA1* expression. Over the past decade, multiple research groups have reported conflicting findings concerning the functional roles of *HTRA1* rs11200638 and ARMS2 in/del mutations in relation to the transcriptional regulation of *HTRA1*.

## Age-related Macular Degeneration Risk Variant *rs11200638* and *HTRA1*

In 2006, the homozygous risk genotype AA at rs1200638 was initially found to be strongly associated with higher levels of *HTRA1* mRNA and protein in blood lymphocytes and RPE cells from four AMD patients compared to three controls without the risk variant.[50] This correlation was subsequently confirmed in archived eye tissues and fresh placenta tissues through RT-qPCR and immunohistochemistry.[51-54] It was hypothesized that this correlation could be attributed to the disruption of a CpG island, which removed inhibitory effects on the methylated CpG island, leading to altered transcription of *HTRA1* or increased responsiveness of the *HTRA1* gene to microenvironmental factors.[55,56] However, subsequent studies have yielded conflicting results, with several investigations suggesting that the SNP rs11200638 is not related to *HTRA1* transcription in the retina or lymphocytes.[30,52,57]

*In vitro* studies have also produced varying results. Dewan *et al*. observed that the risk homozygote (AA) genotype led to a persistent trend of higher luciferase expression than the GG genotype in ARPE-19 and HeLa S3 cells.[58] However, the following year, Kanda *et al*. found no significant difference in luciferase activity associated with rs1200638 in HEK 293, ARPE-19, and Y79 cells.[30] Recently, He *et al*. discovered that the risk SNP rs1200638 significantly increased the responsiveness of the *HTRA1* promoter to oxidative stress and inflammatory responses, resulting in elevated *HTRA1* expression in ARPE-19 cells.[59] These discordant results regarding the effect of rs1200638 on *HTRA1* transcription may be attributed to cell type-dependent effects *in vitro*. In a recent study, Chang *et al*. isolated the effect of rs1200638 using CRISPR manipulation of induced pluripotent stem cell (iPSC)-RPE cells and found that the risk SNP rs1200638 significantly increased *HTRA1* transcription and expression, as confirmed by RT-qPCR and Western blot (WB) analysis, respectively.[60]

## Age-related Macular Degeneration Risk Variant *ARMS2 in/del*

Researchers have also explored the impact of the in/del mutation on *HTRA1* expression. In 2010, Yang *et al*. observed that the haplotype tagged by the in/del mutation led to an upregulation of *HTRA1* transcription in human and mouse RPE cells using luciferase constructs.[54] However, in 2011, Friedrich *et al*. failed to detect this upregulation in ARPE-19 and rMC-1 cells.[52] To validate the regulatory effect, in 2015, our group performed transcription regulator activity assays using luciferase constructs containing the −4320 to +1 base pair region of the non-in/del regulatory region and the −3936 to +1 base pair region of the in/del regulatory region.[61] The transcription was significantly upregulated with in/del variant in both 661W and RGC5 cells but not in ARPE-19 cells, indicating cell type-dependent effects on *HTRA1* transcriptional regulation. In 2018, Oura *et al*. replicated this upregulation in Y79 human retinoblastoma cells.[48]

To identify the activator that binds to the in/del region, we performed gel electrophoresis mobility shift assays and liquid chromatography-mass spectrometry using nuclear extracts from 661W cells. Among the listed in/del interacting proteins, general transcription factor IIi (Gtf2i) was identified with most number of peptide hits and was the sole transcription factor protein. In addition, Gtf2i commonly targets the c-fos cis-element-binding nucleotide sequence, which was found within the in/del sequence.[62] We further validated that Gtf2i bound to the in/del region can enhance *HTRA1* expression through WB and enzyme-linked immunosorbent assay in transfected 661W cell lines.[62] This discovery provides new insights into the molecular mechanism of *HTRA1* transcription regulated by the highly AMD-associated in/del haplotype.

## *HTRA1* Expression in Human Samples

### Induced pluripotent stem cells derived from age-related macular degeneration patients

Since 2007, human iPSCs have rapidly become a valuable investigative tool for gaining insights into the pathology and cellular biology of retinal diseases.[63] In 2015, to measure the *HTRA1* expression levels in AMD patients, our group generated human iPSCs, revealing a significant increase of *HTRA1* mRNA in both

heterozygous and homozygous in/del-AMD iPSCs compared to nonrisk controls.[61] Three years later, Lin *et al*. observed a similar elevation of *HTRA1* expression in iPSC-RPE cells from heterozygous in/del-AMD compared to controls.[64] Recently, Chang *et al*. also replicated the increase of *HTRA1* expression in iPSC-RPE cells derived from AMD patients carrying the *ARMS2/HTRA1* risk variants. Furthermore, they isolated the effects of the individual SNV using CRISPR and found that both *ARMS2* rs10490924 and *HTRA1* rs11200638 independently elevated *HTRA1* expression.[60]

### *HTRA1* in serum/plasma or aqueous humor samples

Since *HTRA1* is known as a widely secreted serine protease, we investigated the relationship between blood circulation *HTRA1* levels in plasma/serum and AMD. We compared 478 AMD patients to 481 controls from diverse geographical regions, including Japan, India, Australia, and the USA. Our findings revealed a statistically significant increase of *HTRA1* in serum or plasma in AMD cases compared with controls.[62] Notably, we observed a consistent rise in *HTRA1* levels with age in controls but not in AMD cases.[62] Recently, this trend was further verified in human retina samples.[26] In addition, we identified that in/del variant increased the secretion of *HTRA1* in both plasma and serum samples.[62] Our results align with the findings of Qureshi and Ambreen, who conducted a similar investigation using 190 serum samples from Pakistan and detected significantly elevated serum *HTRA1* levels in AMD patients.[65] Furthermore, Tosi *et al*. measured *HTRA1* concentrations in the aqueous humor of nAMD patients and found that it was significantly higher than that in controls.[66] These collective observations strengthen the association between circulating *HTRA1* levels and AMD, emphasizing the potential utility of *HTRA1* as a biomarker for AMD.

### *HTRA1* in retina tissue

To improve the accuracy of *HTRA1* expression levels in association with AMD risk, several other research groups have investigated *HTRA1* expression in human retinal tissues. These reports presented varying conclusions regarding *HTRA1* expression in human retina tissues, which can be categorized as follows: (1) elevated *HTRA1* levels in AMD retina (positive),[50,51,53,67] (2) reduced *HTRA1* levels in AMD retina (negative),[30,36] and (3) no significant association.[68] These divergent findings could be attributed to the limited sample sizes used in these studies. Currently, the largest published sample size for AMD research consists of only 18 cases. Given that AMD is a multifactorial disease influenced by factors such as advanced age, environmental variables, genetic variations, and other yet-to-be-discovered factors, the current number of AMD retinal samples in published studies may not be sufficient to establish the definite relationship between *HTRA1* expression level and AMD risk. Therefore, future research should be undertaken to thoroughly investigate *HTRA1* expression in the retinas of AMD patients with age-matched controls.

## Conclusion

AMD is a complex multifactorial disease with contributions from various risk factors. Human genetics research has illuminated the pivotal pathways involved in AMD pathogenesis. Notably, numerous recent genetic studies have underscored the importance of the *ARMS2/HTRA1* locus as one of the strongest genetic factors contributing to AMD onset. The compelling association between AMD and *HTRA1* expression upregulated by *ARMS2/HTRA1* risk variants has progressed to developing *HTRA1* inhibitors as potential drug targets. To date, clinical trials targeting *HTRA1* in AMD have shown limited success, possibly due to the intricate interplay of multiple risk factors affecting AMD. Therefore, a comprehensive approach, encompassing diverse perspectives, integrating various technologies, and fostering multidisciplinary collaboration among experts, is essential for a thorough understanding of AMD mechanisms.

### Ethical statement

The Institutional Review Board of NHO Tokyo Medical Center (No. R21-120) granted approval for this study.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

### Data availability statement

The datasets generated during and/or analyzed during the current study are publicly available.


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## Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

## References


1. Fleckenstein M, Keenan TD, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, *et al.* Age-related macular degeneration. Nat Rev Dis Primers 2021;7:31.
2. Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, *et al.* Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis. Lancet Glob Health 2014;2:e106-16.
3. Fleckenstein M, Mitchell P, Freund KB, Sadda S, Holz FG, Brittain C, *et al.* The progression of geographic atrophy secondary to age-related macular degeneration. Ophthalmology 2018;125:369-90.
4. Spaide RF, Jaffe GJ, Sarraf D, Freund KB, Sadda SR, Staurenghi G, *et al.* Consensus nomenclature for reporting neovascular age-related macular degeneration data: Consensus on neovascular age-related macular degeneration nomenclature study group. ophthalmology 2020;127:616-36.
5. Klein R, Li X, Kuo JZ, Klein BE, Cotch MF, Wong TY, *et al.* Associations of candidate genes to age-related macular degeneration among racial/ethnic groups in the multi-ethnic study of atherosclerosis. Am J Ophthalmol 2013;156:1010-20.e1.
6. Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. Age-related macular degeneration: Genetics and biology coming together. Annu Rev Genomics Hum Genet 2014;15:151-71.
7. Rim TH, Kawasaki R, Tham YC, Kang SW, Ruamviboonsuk P, Bikbov MM, *et al.* Prevalence and pattern of geographic atrophy in Asia: The Asian eye epidemiology consortium. Ophthalmology 2020;127:1371-81.
8. Sobrin L, Seddon JM. Nature and nurture- genes and environment- predict onset and progression of macular degeneration. Prog Retin Eye Res 2014;40:1-15.
9. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, *et al.* Complement factor H variant increases the risk of age-related macular degeneration. Science 2005;308:419-21.
10. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, *et al.* Complement factor H polymorphism in age-related macular degeneration. Science 2005;308:385-9.
11. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005;308:421-4.
12. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, *et al*. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci U S A 2005;102:7227-32.
13. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet 2005;77:389-407.
14. Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, *et al.* Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet 2005;14:3227-36.
15. Rudnicka AR, Jarrar Z, Wormald R, Cook DG, Fletcher A, Owen CG. Age and gender variations in age-related macular degeneration prevalence in populations of European ancestry: A meta-analysis. Ophthalmology 2012;119:571-80.
16. Okamoto H, Umeda S, Obazawa M, Minami M, Noda T, Mizota A, *et al.* Complement factor H polymorphisms in Japanese population with age-related macular degeneration. Mol Vis 2006;12:156-8.
17. Yoshida T, DeWan A, Zhang H, Sakamoto R, Okamoto H, Minami M, *et al.* HTRA1 promoter polymorphism predisposes Japanese to age-related macular degeneration. Mol Vis 2007;13:545-8.
18. Seddon JM, Sharma S, Adelman RA. Evaluation of the clinical age-related maculopathy staging system. Ophthalmology 2006;113:260-6.
19. Goto A, Akahori M, Okamoto H, Minami M, Terauchi N, Haruhata Y, *et al.* Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population. J Ocul Biol Dis Infor 2009;2:164-75.
20. Palkar AH, Khetan V. Polypoidal choroidal vasculopathy: An update on current management and review of literature. Taiwan J Ophthalmol 2019;9:72-92.
21. Xu Y, Guan N, Xu J, Yang X, Ma K, Zhou H, *et al.* Association of CFH, LOC387715, and HTRA1 polymorphisms with exudative age-related macular degeneration in a northern Chinese population. Mol Vis 2008;14:1373-81.
22. Ng TK, Chen LJ, Liu DT, Tam PO, Chan WM, Liu K, *et al.* Multiple gene polymorphisms in the complement factor H gene are associated with exudative age-related macular degeneration in Chinese. Invest Ophthalmol Vis Sci 2008;49:3312-7.
23. Chen LJ, Liu DT, Tam PO, Chan WM, Liu K, Chong KK, *et al.* Association of complement factor H polymorphisms with exudative age-related macular degeneration. Mol Vis 2006;12:1536-42.
24. Kim NR, Kang JH, Kwon OW, Lee SJ, Oh JH, Chin HS. Association between complement factor H gene polymorphisms and neovascular age-related macular degeneration in Koreans. Invest Ophthalmol Vis Sci 2008;49:2071-6.
25. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, *et al.* A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet 2016;48:134-43.
26. Pan Y, Fu Y, Baird PN, Guymer RH, Das T, Iwata T. Exploring the contribution of ARMS2 and HTRA1 genetic risk factors in age-related macular degeneration. Prog Retin Eye Res 2023;97:101159.
27. Uffelmann E, Huang QQ, Munung NS, de Vries J, Okada Y, Martin AR, *et al.* Genome-wide association studies. Nat Rev Methods Primers 2021;1:59.
28. Kortvely E, Hauck SM, Behler J, Ho N, Ueffing M. The unconventional secretion of ARMS2. Hum Mol Genet 2016;25:3143-51.
29. May A, Su F, Dinh B, Ehlen R, Tran C, Adivikolanu H, *et al.* Ongoing controversies and recent insights of the ARMS2-HTRA1 locus in age-related macular degeneration. Exp Eye Res 2021;210:108605.
30. Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, *et al*. A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci U S A 2007;104:16227-32.
31. Wang G, Spencer KL, Court BL, Olson LM, Scott WK, Haines JL, *et al.* Localization of age-related macular degeneration-associated

ARMS2 in cytosol, not mitochondria. Invest Ophthalmol Vis Sci 2009;50:3084-90.
32. Kortvely E, Hauck SM, Duetsch G, Gloeckner CJ, Kremmer E, Alge-Priglinger CS, *et al.* ARMS2 is a constituent of the extracellular matrix providing a link between familial and sporadic age-related macular degenerations. Invest Ophthalmol Vis Sci 2010;51:79-88.
33. Nakayama M, Iejima D, Akahori M, Kamei J, Goto A, Iwata T. Overexpression of HtrA1 and exposure to mainstream cigarette smoke leads to choroidal neovascularization and subretinal deposits in aged mice. Invest Ophthalmol Vis Sci 2014;55:6514-23.
34. Liu J, Hoh J. Postnatal overexpression of the human ARMS2 gene does not induce abnormalities in retina and choroid in transgenic mouse models. Invest Ophthalmol Vis Sci 2015;56:1387-8.
35. Kortvely E, Ueffing M. Gene structure of the 10q26 Locus: A clue to cracking the ARMS2/HTRA1 Riddle? Adv Exp Med Biol 2016;854:23-9.
36. Williams BL, Seager NA, Gardiner JD, Pappas CM, Cronin MC, Amat di San Filippo C, *et al.* Chromosome 10q26-driven age-related macular degeneration is associated with reduced levels of HTRA1 in human retinal pigment epithelium. Proc Natl Acad Sci U S A 2021;118:e2103617118.
37. Clausen T, Kaiser M, Huber R, Ehrmann M. HTRA proteases: Regulated proteolysis in protein quality control. Nat Rev Mol Cell Biol 2011;12:152-62.
38. Kato T, Manabe RI, Igarashi H, Kametani F, Hirokawa S, Sekine Y, *et al.* Candesartan prevents arteriopathy progression in cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy model. J Clin Invest 2021;131:e140555.
39. Chien J, Aletti G, Baldi A, Catalano V, Muretto P, Keeney GL, *et al.* Serine protease HtrA1 modulates chemotherapy-induced cytotoxicity. J Clin Invest 2006;116:1994-2004.
40. Tsuchiya A, Yano M, Tocharus J, Kojima H, Fukumoto M, Kawaichi M, *et al.* Expression of mouse HtrA1 serine protease in normal bone and cartilage and its upregulation in joint cartilage damaged by experimental arthritis. Bone 2005;37:323-36.
41. Akahori M, Oka C, Iwata T. Characterization of the Retina in Htra1 deficient mouse. Invest Ophthalmol Vis Sci 2008;49:3418.
42. Jones A, Kumar S, Zhang N, Tong Z, Yang JH, Watt C, *et al.* Increased expression of multifunctional serine protease, HTRA1, in retinal pigment epithelium induces polypoidal choroidal vasculopathy in mice. Proc Natl Acad Sci U S A 2011;108:14578-83.
43. Zhang L, Lim SL, Du H, Zhang M, Kozak I, Hannum G, *et al.* High temperature requirement factor A1 (HTRA1) gene regulates angiogenesis through transforming growth factor-β family member growth differentiation factor 6. J Biol Chem 2012;287:1520-6.
44. Ahamed W, Yu RM, Pan Y, Iwata T, Barathi VA, Wey YS, *et al.* HTRA1 regulates subclinical inflammation and activates proangiogenic response in the retina and choroid. Int J Mol Sci 2022;23:10206.
45. Vierkotten S, Muether PS, Fauser S. Overexpression of HTRA1 leads to ultrastructural changes in the elastic layer of Bruch's membrane via cleavage of extracellular matrix components. PLoS One 2011;6:e22959.
46. Kumar S, Berriochoa Z, Ambati BK, Fu Y. Angiographic features of transgenic mice with increased expression of human serine protease HTRA1 in retinal pigment epithelium. Invest Ophthalmol Vis Sci 2014;55:3842-50.
47. Kumar S, Nakashizuka H, Jones A, Lambert A, Zhao X, Shen M, *et al.* Proteolytic degradation and inflammation play critical roles in polypoidal choroidal vasculopathy. Am J Pathol 2017;187:2841-57.
48. Oura Y, Nakamura M, Takigawa T, Fukushima Y, Wakabayashi T, Tsujikawa M, *et al.* High-temperature requirement A 1 causes photoreceptor cell death in zebrafish disease models. Am J Pathol 2018;188:2729-44.
49. Grassmann F, Heid IM, Weber BH, International AMD Genomics Consortium (IAMDGC). Recombinant haplotypes narrow the ARMS2/HTRA1 association signal for age-related macular degeneration. Genetics 2017;205:919-24.
50. Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, *et al.* A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science 2006;314:992-3.
51. Chan CC, Shen D, Zhou M, Ross RJ, Ding X, Zhang K, *et al.* Human HtrA1 in the archived eyes with age-related macular degeneration. Trans Am Ophthalmol Soc 2007;105:92-7.
52. Mohamad NA, Ramachandran V, Mohd Isa H, Chan YM, Ngah NF, Ching SM, *et al.* Association of HTRA1 and ARMS2 gene polymorphisms with response to intravitreal ranibizumab among neovascular age-related macular degenerative subjects. Hum Genomics 2019;13:13.
53. Tuo J, Ross RJ, Reed GF, Yan Q, Wang JJ, Bojanowski CM, *et al.* The HtrA1 promoter polymorphism, smoking, and age-related macular degeneration in multiple case-control samples. Ophthalmology 2008;115:1891-8.
54. Yang Z, Tong Z, Chen Y, Zeng J, Lu F, Sun X, *et al.* Genetic and functional dissection of HTRA1 and LOC387715 in age-related macular degeneration. PLoS Genet 2010;6:e1000836.
55. Wang G. Chromosome 10q26 locus and age-related macular degeneration: A progress update. Exp Eye Res 2014;119:1-7.
56. Friedrich U, Myers CA, Fritsche LG, Milenkovich A, Wolf A, Corbo JC, *et al.* Risk- and non-risk-associated variants at the 10q26 AMD locus influence ARMS2 mRNA expression but exclude pathogenic effects due to protein deficiency. Hum Mol Genet 2011;20:1387-99.
57. Chowers I, Meir T, Lederman M, Goldenberg-Cohen N, Cohen Y, Banin E, *et al.* Sequence variants in HTRA1 and LOC387715/ARMS2 and phenotype and response to photodynamic therapy in neovascular age-related macular degeneration in populations from Israel. Mol Vis 2008;14:2263-71.
58. Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, *et al.* HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 2006;314:989-92.
59. He F, Li X, Cai S, Lu L, Zhang T, Yang M, *et al.* Polymorphism rs11200638 enhanced HtrA1 responsiveness and expression are associated with age-related macular degeneration. Eye (Lond) 2022;36:1631-8.
60. Chang YJ, Jenny LA, Li YS, Cui X, Kong Y, Li Y, *et al.* CRISPR editing demonstrates rs10490924 raised oxidative stress in iPSC-derived retinal cells from patients with ARMS2/HTRA1-related AMD. Proc Natl Acad Sci U S A 2023;120:e2215005120.
61. Iejima D, Itabashi T, Kawamura Y, Noda T, Yuasa S, Fukuda K, *et al.* HTRA1 (high temperature requirement A serine peptidase 1) gene is transcriptionally regulated by insertion/deletion nucleotides located at the 3′ end of the ARMS2 (age-related maculopathy susceptibility 2) gene in patients with age-related macular degeneration. J Biol Chem 2015;290:2784-97.
62. Pan Y, Iejima D, Nakayama M, Suga A, Noda T, Kaur I, *et al.* Binding of Gtf2i-β/δ transcription factors to the ARMS2 gene leads to increased circulating HTRA1 in AMD patients and *in vitro*. J Biol Chem 2021;296:100456.
63. Fields MA, Bowrey HE, Gong J, Moreira EF, Cai H, Del Priore LV. Extracellular matrix nitration alters growth factor release and activates bioactive complement in human retinal pigment epithelial cells. PLoS One 2017;12:e0177763.
64. Lin MK, Yang J, Hsu CW, Gore A, Bassuk AG, Brown LM, *et al.* HTRA1, an age-related macular degeneration protease, processes extracellular matrix proteins EFEMP1 and TSP1. Aging Cell 2018;17:e12710.
65. Qureshi IZ, Ambreen F. Serum APOE, leptin, CFH and HTRA1

levels in Pakistani age related macular degeneration patients. J Pak Med Assoc 2017;67:852-7.
66. Tosi GM, Caldi E, Neri G, Nuti E, Marigliani D, Baiocchi S, *et al.* HTRA1 and TGF-β1 concentrations in the aqueous humor of patients with neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci 2017;58:162-7.
67. Melo E, Oertle P, Trepp C, Meistermann H, Burgoyne T, Sborgi L, *et al.* HtrA1 mediated intracellular effects on tubulin using a polarized RPE disease model. EBioMedicine 2018;27:258-74.
68. Kanda A, Stambolian D, Chen W, Curcio CA, Abecasis GR, Swaroop A. Age-related macular degeneration-associated variants at chromosome 10q26 do not significantly alter ARMS2 and HTRA1 transcript levels in the human retina. Mol Vis 2010;16:1317-23.