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Association of *ARMS2, HTRA1* and *CFH* genes polymorphisms in patients with age-related macular degeneration in the Malaysian population

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Abstract

Background Despite extensive research efforts, understanding the precise causes and molecular underpinnings of age-related macular degeneration (AMD) remains elusive. Exploring different populations becomes crucial to establish conclusive insights into the role of genetic factors in AMD.

Methodology This study aimed to investigate the association between the well-documented major risk alleles in the *HTRA1, ARMS2* and *CFH* genes with AMD in the Malaysian multi-ethnic population. A total of 205 subjects were enrolled in this study, 103 were diagnosed with AMD while 102 represented the control subjects. Genomic DNA was extracted from peripheral blood mononuclear cells and gene amplification was performed by polymerase chain reaction. Subsequently, genotyping for the *HTRA1*, *ARMS2* and *CFH* genes was performed using direct DNA sequencing analysis.

Results Significant associations ($p < 0.05$) were detected with AMD for both SNP rs11200638: G > A in the promoter of *HTRA1* and rs10490924: G > T in *ARMS2* but not for variant Y402H in *CFH* gene ($p > 0.05$) in our study population. The A allele frequency of rs11200638 in the *HTRA1* promoter was 51.9% in cases versus 39.2% in controls ($p = 0.010$). The frequency of AA genotype was 28.2% for AMD cases, compared to 17.6% in controls (OR 2.58, 95% CI 1.19–5.58; $p = 0.043$). The frequency of the TT genotype of rs10490924 in *ARMS2* was 25.2% in cases versus 8.8% in controls (OR 2.23, 95% CI 0.83–5.99; $p = 0.002$).

Conclusion The study reveals an association between specific genetic variants in the *HTRA1* and *ARMS2* genes and the occurrence of AMD in the Malaysian population. However, contrary to expectations, the study did not identify a substantial correlation between AMD and the Y402H variant of the *CFH* gene in this specific population.

Keywords Age-related macular degeneration, *HTRA1* gene, *ARMS2* gene, *CFH* gene, Single nucleotide polymorphism

Background

Age-related macular degeneration (AMD) is a complex, progressive disease which influenced by both genetic and environmental factors, yet its exact cause remains elusive despite extensive research [1, 2]. With an anticipated demographic shift towards aging populations, the prevalence of AMD is set to double in the coming years, posing a growing concern for older Malaysians [3]. Notably, the age-standardized prevalence of AMD in a multi-ethnic

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Asian cohort, comprising Singaporean Chinese (7.3%), Indian (5.7%), and Malay (7.7%) ethnic groups, exhibited similar rates [4].

AMD profoundly impacts both individuals and society at large, particularly among older adults, leading to significant vision loss and diminished quality of life. The economic implications of AMD are substantial, with high healthcare costs for ongoing medical care and assistive technologies, coupled with indirect costs from reduced workforce participation and productivity losses. Caregivers bear additional burdens, and the condition is associated with heightened rates of depression and anxiety, necessitating mental health support. The increasing prevalence of AMD also strains public health resources, emphasizing the crucial need for educational programs, early detection initiatives, and effective treatments. Therefore, addressing AMD in Malaysia requires a comprehensive approach, including regular screenings, promotion of healthy lifestyles, and continual research to enhance treatment options. Collaborative efforts shall be aimed at increasing awareness, improving access to care, and effectively managing AMD, thereby safeguarding vision and the quality of life of aging Malaysians.

Genes in the complement pathway and a region of chromosome 10 have been implicated as the major genetic contributors to AMD. *Complement Factor H* (*CFH*) gene is located on the q arm of chromosome 1 at position 32 with the Tyr402His polymorphism was one of the variants identified. CFH usually has the amino acid tyrosine (Tyr) at position 402, but occasionally it may contain the amino acid histidine (His) instead. This version is less effective at regulating the complement system than the version with Tyr at position 402, which may help explain the increased disease risk. Studies assessing the association between the *CFH* Tyr402His polymorphism and AMD have indicated that this polymorphism plays a role in the majority of AMD cases in the Caucasian population [5] than in the Asian cohorts.

Both *Human High-Temperature Requirement A-1* (*HTRA1*) and *Age-Related Maculopathy Susceptibility 2* (*ARMS2*) genes are in chromosome 10 on the q arm at positions 26.3 and 26.13, respectively. *HTRA1* gene is reported as a likely causal variant for AMD with a population-attributed risk of 49.3% and 86% in the Caucasian [6] and Chinese populations [7], respectively. A functional HTRA1 protein is necessary for the cellular and environmental stress damage control system in *Escherichia coli* [8]. Aside from this, the expression *HTRA1* gene was also found to be activated under oxidative stress thus, offering a protective barrier from cell death but at the same time, boosting early senescence [9]. This functional property of the *HTRA1* gene could explain the enhanced expression of this protein in conditions relating to both wet and dry AMD lesions in the older age group [10].

ARMS2 gene encodes a hypothetical protein whose function may very likely be involved in the complement system, thus mediating the clearance of cellular debris due to apoptosis and/or necrosis [11]. It is highly expressed in placental and retinal tissues. A single-nucleotide polymorphism (SNP) known as rs10490924, maps to exon 1 of the *ARMS2* gene changes putative amino acid 69 from alanine to serine (A69S) which alters the ARMS2 protein [12]. Notably, ARMS2 directly interacts with fibulin-6 (hemicentin-1) and mutations in the *fibulin-6* gene were previously demonstrated to cause familial AMD [13].

Therefore, it is imperative to conduct studies in different populations to draw firm conclusions about the role of genetic factors in AMD. This prompted us to investigate whether the previously reported major risk alleles in the *HTRA1, ARMS2* and *CFH* genes are associated with AMD in the multi-ethnic Malaysian population.

Subjects, materials and methods

Subject selection and recruitment

This study was approved by the Universiti Malaya Medical Centre (UMMC) Ethical Committee (Institutional Review Board), Kuala Lumpur, Malaysia, following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use—Good Clinical Practice guideline and the Declaration of Helsinki (Reference No.: 757.48). Written informed consent was obtained from patients before they were enrolled on this study. Two groups of 103 patients with AMD and 102 control subjects were recruited in the duration of 12 months (Jan–Dec 2011) from the outpatient clinic of the Department of Ophthalmology, UMMC. All subjects were examined by a retinal surgeon and were subjected to a standard ophthalmic examination, including visual acuity measurement, slit lamp biomicroscopy, and dilated fundi examination. Coloured fundoscopy photos were taken for all these subjects and were interpreted by a second retinal surgeon for the AMD categories to avoid operator-dependent variability. Patients with confirmed cases of AMD based on a standardized set of diagnostic criteria established by the Age-Related Eye Disease Study (AREDS) categories 2, 3 and 4 were included in the study [14].

Briefly, those patients who have early stage of the disease with multiple small drusen, single or non-extensive intermediate drusen (63–124 μm), RPE pigmentary abnormalities, or any combination of these in one or both eyes and visual acuity of 20/30 or better in both eyes is considered as AMD category 2. While those patients who exhibited mid-stage disease with at least

one eye having visual acuity of 20/30 or better and one large drusen (125 μm), extensive intermediate drusen, or geographic atrophy that did not involve the macula or any combination of these is considered AMD category 3. On the other hand, those patients with advanced AMD with substantial choroidal neovascularisation or geographic atrophy involving the macula in one or both eyes are considered AMD category 4. Further, patients who had AMD fulfilling category 4 by the AREDS had fluorescein and indocyanine green fundus angiography. On this basis, those individuals without evident signs of AMD, displaying no drusen, pigmentary abnormalities, or other notable retinal changes, and has a visual acuity of 20/30 or better in both eyes and consequently, were excluded from the study.

While the control cohort consisted of age-matched volunteers without visual impairment, no family history of AMD and no macular drusen or exhibited any clinical evidence of retinal disorder. Exclusion criteria for both cohorts include subjects with any eye disease, retinopathy, or media opacities. Further, subjects with mixed ancestry were excluded from the study to minimise the population stratification effects. In this cohort, the individuals of Indian descent exclusively belonged to the South Indian ancestry group.

Genotyping analysis of the three SNPs: HTRA1 rs11200638, ARMS2 rs10490924 and CFH rs1061170

Blood samples were collected from all participants and the genomic DNA was extracted from the peripheral venous blood of each participant using QIAamp®DNA Minikit (Qiagen, Germany) according to the manufacturer's protocol. PCR reactions were performed using a PCR thermal cycler (Biometra, Germany) in 50 μl PCR reaction tubes containing 100–250 ng of genomic DNA, 1X *Taq* buffer with KCl, 2.5 mM of MgCl, 200 μM of dNTPs, 8% dimethylsulfoxide, 1 unit of *Taq* DNA polymerase (Fermentas, USA) and 20 pmol of each forward and reverse primers. All primer sequences for the *HTRA1, ARMS2* and *CFH* genes were sourced from Xu et al. [15]. Thirty-five cycles of amplification were carried out with standard PCR protocol at an annealing temperature of 55 °C for *HTRA1* and *ARMS2* and 53 °C for *CFH* gene for all reactions for 30 s. Direct DNA sequencing of the *HTRA1, ARMS2* and *CFH* genes was carried out using ABI Prism Gene Sequencer, Model 3100, version 3.7 at Research Biolabs in Singapore, in duplicates. The genotyping process was conducted with the individuals deliberately unaware of the sample identities, ensuring a blinded approach to minimize bias or influence during the genotyping procedures.

Data analyses

The genetic Hardy–Weinberg equilibrium (HWE) of *HTRA1, ARMS2* and *CFH* genes in cases and controls were calculated using Genepop version 4.2 population genetics software package [16]. The Statistical Package for Social Sciences version 26.0 (IBM, New York, USA) was used to analyse the data. One-sample Kolmogorov–Smirnov test was used to assess that a variable is normally distributed. The student's *t-test* was used to compare means between the cases and controls for all data sets that showed normal distribution. Chi-square (X^2) and/or Fisher's exact tests were used for association studies between the *HTRA1, ARMS2* and *CFH* gene polymorphisms and susceptibility to AMD, where applicable. Odds ratios (OR) and 95% confidence intervals (CI) were calculated as a relative measure of effect and the level of uncertainty around the measure of effect, respectively. A p value of less than 0.05 was considered statistically significant. The parameters for power calculation for *HTRA1* and *ARMS2* association studies, respectively, were set as follows using genetic power calculator [17].

High-risk allele frequency A/T set at	0.5
Prevalence set at	7%
Genotype relative risk GA/GT	1
Genotype relative risk AA/TT	3
D-prime	1
Marker allele frequency G	0.5
Number of cases	100
Control: case ratio	1
Type I error	0.05
Required sample size at power	0.80

With that, the power for *HTRA1* and *ARMS2* chi-squared tests were 95% each, at 5% Type I error, using 100 cases and 100 controls.

Results

A total of 205 subjects were enrolled on this study; $n=103$ subjects were with AMD whilst, $n=102$ subjects represent the age-matched control group (Table 1). There was no significant association between groups of subjects and gender as well as ethnicity (Table 1). Additionally, upon employing the AREDS-based criteria [14] for the classification of AMD subjects, it was similarly observed that there was no statistically significant association between the groups of subjects and their respective ethnicities (Table 2).

The genotype and allele frequencies of the *HTRA1, ARMS2* and *CFH* gene polymorphism among subjects with AMD and controls are tabulated in Table 3. Genotype distributions for SNPs at gene loci *HTRA1, ARMS2*

Table 1 Demographic characteristics of patients with AMD and control subjects

Characteristics	AMD Cases ($n=103$)	Controls ($n=102$)	p value
Age (year)[1] [Range]	73.7 ± 7.6 [49–93]	72.8 ± 7.7 [49–93]	0.388
Gender[2]			
Male	52	47	0.528 ($\chi^2=0.399$)
Female	51	54	
Ethnicity[2]			
Chinese	53	53	0.990 ($\chi^2=0.021$)
Indian	30	30	
Malay	20	19	

[1] Data were expressed in means ± SD. The data set was normally distributed ($p>0.05$, one-sample Kolmogorov–Smirnov test) therefore, an independent t-test was used for the test of mean differences

[2] Association study was performed using χ^2 test

and *CFH* were in HWE (Table 3). The SNPs rs11200638: G > A in *HTRA1* and rs10490924: G > T in *ARMS2* promoter regions were significantly associated with AMD. The frequencies of the risk allele A in the former SNP were found significantly higher among those with AMD cases compared to controls whilst, similar risk T allele frequencies were observed in the *ARMS2* gene (Table 3). A significant difference was found in the *HTRA1* and *ARMS2* genotype frequencies between AMD cases and controls (Table 3). On the other hand, no differences were observed in the genotypic and allelic frequencies of *CFH* variant Y402H between the groups of subjects (Table 3).

Table 4 demonstrates a joint genotypes association testing by using 9 possible combinations of *ARMS2* and (followed by) *HTRA1* genotypes in the groups of subjects demarcated according to the ethnicities of the subjects. The results showed that the genotype combination of TT AA of *ARMS2* and *HTRA1* was significantly associated with the susceptibility to AMD among the Malaysian Chinese and Indian ethnic groups compared to the control subjects, respectively (Table 4). However, the frequencies of combined genotypes of *ARMS2* and *HTRA1* were statistically similar between patients with AMD and controls among the Malaysian Malay ethnic group (Table 4), which may have resulted due to a relatively smaller sample size in this subpopulation.

Figure 1 demonstrates the age profile of patients who have the TT genotype for the *ARMS2* gene and the three possible genotypes AA, GA, and GG for the *HTRA1* locus. Patients with AMD appeared to have mostly AA and GA genotypes, whilst the control subjects, had GG genotypes. Despite being relatively advanced in age, the four control subjects belonging to the Chinese ethnic group who have the GG genotype for the *HTRA1* gene did not develop AMD. Interestingly, we did have a relatively young subject (51 years old) in the control Malaysian Indian ethnic sub-group carrying the TT and AA genotypes for the *ARMS2* and *HTRA1* gene loci. Given the results of the present study that have shown a significant association between the genotypic combination of TT AA of *ARMS2* and *HTRA1* and susceptibility to AMD among the Malaysian Indian ethnic groups, it would be very interesting to follow up if this subject would develop AMD as time progresses.

Discussion

The main objective of this study was to examine if the major risk alleles in the *HTRA1*, *ARMS2* and *CFH* genes were associated with AMD in the multi-ethnic Malaysian population. Wide inter-population variations of alleles of *HTRA1*, *ARMS2* and *CFH* genes were observed, worldwide (Tables 5A-C). The distribution of alleles of *HTRA1*, *ARMS2* and *CFH* genes in the Malaysian pooled population was generally comparable to other Asian populations (Tables 5, 6, and 7), except for a few studies involving *CFH* gene polymorphism [18–20]. The variation of alleles found in *HTRA1*, and *ARMS2* genes observed in the present study were also similarly reported by Mohamed et al. [21], an antecedent study in which the treatment efficacies of ranibizumab among patients with neovascular AMD among Malaysians was examined thus, indicating our study was not biased neither by subject selection nor stratification effects.

Our study showed significant associations between the *HTRA1* and *ARMS2* variants with AMD in the Malaysian population. This is consistent with previous studies published across a wide range of other populations worldwide

Table 2 Classification of AMD subjects by ethnicity using AREDS-based criteria

Ethnicity AREDS classification	Malaysian Chinese ($n=53$)		Malaysian Indian ($n=30$)		Malaysian Malay ($n=20$)		p value
	n	Frequency (%)	n	Frequency (%)	n	Frequency (%)	
Category 2	14	26.4	9	30.0	9	45.0	0.513 ($\chi^2=3.275$)
Category 3	18	34.0	10	33.3	7	35.0	
Category 4	21	39.6	11	36.7	4	30.0	

n Number of cases; *AMD* age-macular degeneration; *AREDS* age-related eye disease study; Categories 2 and 3, early stage AMD; Category 4, late stage AMD. Association study was performed using χ^2 test

Table 3 Frequencies of *HTRA1*, *ARMS2* and *CFH* genotypes and alleles in patients with AMD and controls

SNPs	AMD cases ($n=103$)	Controls ($n=102$)	χ^2	*p* value	OR (95% CI)
HTRA1					
Genotype					
GG	25 (24.3%)	40 (39.2%)	6.300	0.043*	1.0 (reference)
GA	49 (47.6%)	44 (43.1%)			1.78 (0.94–3.39)
AA	29 (28.2%)	18 (17.6%)			2.58 (1.19–5.58)
	HWE, $p=0.70$	HWE, $p=0.41$			
Allele					
G	99 (48.1%)	124 (60.8%)	6.691	0.010*	1.0 (reference)
A	107 (51.9%)	80 (39.2%)			1.68 (1.13–2.48)
ARMS2					
Genotype					
GG	22 (21.4%)	17 (16.7%)	12.260	0.002*	1.0 (reference)
GT	55 (53.4%)	76 (74.5%)			0.56 (0.27–1.15)
TT	26 (25.2%)	9 (8.8%)			2.23 (0.83–5.99)
	HWE, $p=0.56$	HWE, $p=0.37$			
Allele					
G	99 (48.1%)	110 (53.9%)	1.410	0.235	1.0 (reference)
T	107 (51.9%)	94 (46.1%)			1.26 (0.86–1.86)
CFH					
Genotype					
TT	75 (72.8%)	81 (79.4%)	1.226	0.268	1.0 (reference)
TC	28 (27.2%)	21 (20.6%)			1.44 (0.75–2.75)
CC	0 (0%)	0 (0%)	–	–	–
	HWE, $p=0.21$	HWE, $p=0.59$			
Allele					
T	178 (86.4%)	183 (89.7%)	1.060	0.303	1.0 (reference)
C	28 (13.6%)	21 (10.3%)			1.37 (0.75–2.50)

SNPs Single nucleotide polymorphisms; *OR* odds ratio; *HWE* Hardy–Weinberg equilibrium; *HTRA1 Human High-Temperature Requirement A-1; ARMS2 Age-Related Maculopathy Susceptibility 2; CFH Complement Factor H; n* number of cases; Frequencies are shown in parentheses

*$p<0.05$ is considered significant

Table 4 Combination of ARMS2 and HTRA1 genotypes according to AMD status in the multi-ethnic Malaysian population

Genotype combination	Malaysian Chinese			Malaysian Indian			Malaysian Malay		
	AMD ($n=53$)	Control ($n=53$)	*p* value	AMD ($n=30$)	Control ($n=30$)	*p* value	AMD ($n=20$)	Control ($n=19$)	*p* value
GG GG	9	10		1	0		4	4	
GG GA	0	0		1	0		1	0	
GG AA	0	2		0	1		1	0	
TG GG	4	10		2	5		1	3	
TG GA	16	20		15	15		10	9	
TG AA	3	7		5	6		2	1	
TT GG	1	4		2	2		0	2	
TT GA	5	0		1	0		0	0	
TT AA	15	0	0.0001*	3	1	0.03*	1	0	0.66

n number of cases

*$p<0.05$ is considered significant. Association studies were performed using Fisher's exact test

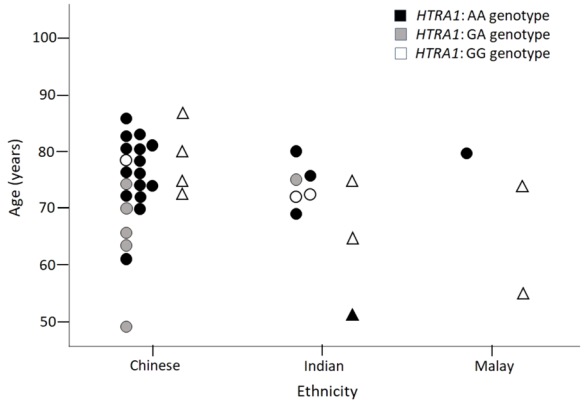

Fig. 1 Age profiles of patients with AMD (circle) and controls (triangle) according to ethnic groups in Malaysia. Those patients with AMD who have the TT genotype for the *ARMS2* gene were further annotated by their *HTRA1* genotypes (AA, GA, and GG)

including the Chinese [15, 19, 25, 27, 40], Korean [26], Japanese [24, 32], Indian [18, 20], Thai [23], Pakistani [33], Caucasian [6, 28–30, 35], Middle Easterners [31, 41], Hispanic [34, 36, 42] and African [34] populations (Tables 5 and 6). Nevertheless, there are indeed subtle differences in the outcome of the analysis in part due to the differences in experimental/study design, subject's inclusion criteria, grading of AMD, etc. in these studies. For example, the definition of cases and controls for the association analysis varies (Tables 5, 6, and 7), as some had categorized the subjects into the early and late stage of AMD or, wet and dry AMD whilst, this study rather took a more generalized view regardless of the stage of presentation. Aside from this, the diagnostic criterion of the present study was based on the AREDS (14, Table 2) whilst few other studies [18, 36, 37] had opted for either Wisconsin Age-Related Maculopathy Grading System, International Age-related Maculopathy Epidemiological Study Group or, Clinical Age-Related Maculopathy System. On the same note, there were also studies with no discernible diagnostic criteria mentioned [6, 20] or genotyping results [22].

ARMS2 and HTRA1 are both located in the chromosome 10q26 region, where the SNP rs11200638 resides (with T allele) in the promoter region of the HTRA1 gene

Table 5 Distribution of allelic frequencies of *HTRA1* gene in the Malaysian population compared to other Asian, Caucasian and Middle Easterners

Population	Allele frequency*		Case and control definition for the association study	Result	References
	G	A			
Asian					
Malaysian pooled	0.61	0.39	AMD versus No AMD	Positive	Present study
Malaysian pooled	0.61	0.39	Advanced AMD versus No AMD	Positive	[21]
Indonesian#	0.69	0.31	–	–	[22]
Indian (Hyderabad)	0.65	0.35	AMD versus No AMD	Positive	[20]
Thai	0.64	0.36	AMD versus No AMD	Positive	[23]
Japanese (Saitama)	0.62	0.38	Wet &/or Dry AMD versus No AMD	Positive	[24]
Northern Chinese (Beijing)	0.58	0.42	Exudative AMD versus No AMD	Positive	[15]
Chinese (Hong Kong)	0.55	0.45	Exudative AMD versus No AMD	Positive	[25]
Chinese (North & South China)	0.55	0.45	Early & Advanced AMD versus No AMD	Positive	[19]
Korean	0.55	0.45	AMD versus No AMD	Positive	[26]
Chinese (Beijing)	0.54	0.46	Exudative AMD versus No AMD	Positive	[27]
Caucasian					
Czechian—Central Europe	0.80	0.20	Advanced AMD versus No AMD	Positive	[28]
Austrian—Central Europe	0.79	0.21	Exudative AMD versus No AMD	Positive	[29]
Non-Hispanic Whites (Utah)	0.75	0.25	AMD versus HC	Positive	[6]
Lithuanian (Northern Europe)	0.72	0.28	Early & Exudative AMD versus No AMD	Positive	[30]
Middle Easterners					
Arabs	0.85	0.15	–	–	[31]
Ashkenazi Jews	0.84	0.16	–	–	[31]
Israelites	0.83	0.17	Exudative AMD versus No AMD	Positive	[31]
Sephardic Jews	0.81	0.19	–	–	[31]

*Data derived from either healthy controls (HC) or the general population;

#Allelic frequency was only available for the patient cohort

Table 6 Distribution of allelic frequencies of *ARMS2* gene in the Malaysian population compared to other Asian, Caucasian, Middle Easterners, Hispanics, African and Mexican

Population	Allele frequency*		Case and control definition for the association study	Result	References
	G	T			
Asian					
Malaysian pooled	0.54	0.46	AMD versus No AMD	Positive	Present study
Malaysian pooled	0.56	0.44	Advanced AMD versus No AMD	Positive	[21]
Indian (North & South India)	0.68	0.32	Early & Late AMD versus No AMD	Positive	[18]
Japanese (Hiroshima)	0.65	0.35	Exudative AMD versus No AMD	Positive	[32]
Indian (Hyderabad)	0.64	0.36	AMD versus No AMD	Positive	[20]
Chinese (Beijing)	0.62	0.38	Exudative AMD versus No AMD	Positive	[27]
Pakistani	0.57	0.43	Wet AMD versus No AMD	Positive	[33]
Northern Chinese (Beijing)	0.57	0.43	Exudative AMD versus No AMD	Positive	[15]
Korean	0.56	0.44	AMD versus No AMD	Positive	[26]
Chinese (North & South China)	0.55	0.45	Early & Advanced AMD versus No AMD	Positive	[19]
Caucasian					
Non-Hispanic Whites (American)	0.81	0.19	AMD versus HC	Positive	[34]
Whites of non-Hispanic descent (Washington)	0.81	0.19	Advanced AMD versus No AMD	Positive	[35]
Czechian—Central Europe	0.79	0.21	Advanced AMD versus No AMD	Positive	[28]
Australian (Sydney)	0.73	0.27	Advanced AMD versus No AMD	Positive	[35]
Middle Easterners					
Ashkenazi Jews	0.81	0.19	–	–	[31]
Israelites—Ashkenazi Jews, Sephardic Jews, Arabs	0.80	0.20	Exudative AMD versus No AMD	Positive	[31]
Arabs	0.80	0.20	–	–	[31]
Sephardic Jews	0.79	0.21	–	–	[31]
Hispanic					
Mexican Mestizo	0.76	0.24	–	–	[36]
Mexican American	0.75	0.25	AMD versus HC	Positive	[34]
Mexican Zapotecos (Amerindian)	0.72	0.28	–	–	[36]
Mexican Mayas (Amerindian)	0.72	0.28	–	–	[36]
Mexican Pooled	0.72	0.28	Advanced AMD versus No AMD	Positive	[36]
African					
African American	0.74	0.26	AMD versus HC	Positive	[34]

*Data derived from either healthy controls (HC) or the general population

(also known as PRSS11, NM_002775) and is approximately 6.1 kb downstream of the ARMS2 rs10490924 locus (with A allele). It was previously reported that both HTRA1 and ARMS2 SNPs appear to contribute equally to the increased disease risk [2]. An analysis by the AMD Gene Consortium reported that both the HTRA1 and the nearby ARMS2 SNP locus are in strong linkage disequilibrium [43]. However, both studies did not state whether the HTRA1 or ARMS2 is the causal gene in this locus. Since these two genes' loci are in proximity, it is difficult to tell whether changes in one gene or another or possibly changes in both accounts for the increased disease risk. Although the polymorphism of Tyr402His in CFH rs1061170 has been identified as a common variant and a major genetic risk factor for AMD development, it did not appear to increase the risk of AMD in this Malaysian cohort. The collaborative genome-wide association study reported stronger evidence of the CFH variant for disease association among Europeans but a lower association among East Asians when stratified based on ancestry [43]. This also explains the consistently low frequencies of the risk-C allele in Asian populations except for a few studies [18–20, 33] (Table 7). In this study, we reported a null association for risk-C allele between the case and control groups of subjects (Table 3) as well as when they were further stratified according to three different Malaysian ethnic subgroups including Chinese as well as Indian and Malay (Table 4).

As observed among the Malaysian Chinese subjects, similar null association results were also reported in

Table 7 Distribution of allelic frequencies of *CFH* gene in the Malaysian population compared to other Asian, Hispanic, Caucasian, African and Middle Easterners

Population	Allele frequency*		Case and control definition for the association study	Result	References
	T	C			
Asian					
Malaysian pooled	0.90	0.10	AMD versus No AMD	Null	Present study
Chinese (Hong Kong)	0.96	0.04	Exudative AMD versus No AMD	Null	[37]
Japanese American (Gifu)	0.93	0.07	–	–	[38]
Northern Chinese (Beijing)	0.92	0.08	Exudative AMD versus No AMD	Null	[15]
Japanese (Hiroshima)	0.91	0.09	Exudative AMD versus No AMD	Null	[32]
North Indian (Chandigarh)	0.83	0.17	Late AMD versus No AMD	Positive	[39]
Hispanic					
Mexican Zapotecos (Amerindian)	0.96	0.04	AMD versus No AMD	Null	[36]
Mexican Mestizo	0.85	0.15	–	–	[36]
Mexican Pooled	0.84	0.16	AMD versus No AMD	Positive	[36]
Mexican American	0.83	0.17	–	–	[38]
Caucasian					
Central Europe (Austria)	0.70	0.30	Exudative AMD versus No AMD	Positive	[29]
Czechian—Central Europe	0.68	0.32	Advanced AMD versus No AMD	Positive	[28]
Whites—Iowa	0.66	0.34	–	–	[38]
African					
Somalis	0.66	0.34	–	–	[38]
African American	0.65	0.35	–	–	[38]
Middle Easterners					
Ashkenazi Jews	0.65	0.35	AMD versus No AMD	Positive	[31]
Israelites—Ashkenazi & Sephardic Jews	0.64	0.36	AMD versus No AMD	Positive	[31]
Sephardic Jews	0.64	0.36	AMD versus No AMD	Null	[31]

*Data derived from either healthy controls (HC) or the general population

multiple other independent studies conducted in China [15, 37] and Japan [32, 44]. In addition, in agreement with the hypothesized significant ethnic differences in minor allele frequencies of Tyr402His [38], this variant was also not associated with AMD in the Mexican Zapotecos individuals [36], who also showed similar frequencies of C risk-allele (0.04) as that of the Japanese [45] and Chinese [37] populations. In contrast, Quan et al. [46] reported strong evidence for an association between *CFH* gene polymorphism and AMD in the Chinese population with a pooled C allele frequency of 0.05, via a meta-analysis study. Albeit studying a similar cohort of Chinese, contrasting results of the association study between the investigations of Chen et al. [37] and Quan et al. [46] is very likely due to the inclusion of subjects from various geographic areas of China comprised of 56 different ethnicities in the latter study, hence, a diverse genetic background. Aside from this, sample size, as well as the sampling frame, may also play a role in such inconclusive results [4].

In contrast to the null association between the *CFH* gene and AMD in the Malaysian Indian cohort found in this study (data not shown), Sharma et al. [39] and Kaur et al. [20] on the other hand, had reported otherwise among subjects with late stages of AMD in the North and South Indian population, respectively. In another study, Sundaresan et al. [18] investigated by including subjects hailing from the North and South regions of India with both early and late stages of AMD. Consistent with the prior-mentioned studies [20, 39], in this study too, an association was found between the *CFH* gene polymorphism and late stage of AMD. When the Malaysian Indian ethnic subpopulation harbouring AMD was further demarcated into early and late stages of AMD, we found that 19 subjects were diagnosed with an early stage of AMD (categories 2 and 3 of AREDS) whilst the remaining 11, are those with late stage (category 4 of AREDS, Table 2). Hence, we postulated that the lack of association between AMD and CFH gene polymorphism in the Malaysian Indian cohort in the present study might be due to the presence of the majority of subjects with early-stage of AMD. Nonetheless, the absence of an association with *CFH* gene polymorphism within the Malaysian Indian population is highly suggestive of alternative

molecular pathways for the development of early AMD when compared with the Caucasian population.

Limitations of the study

A small sample size is the main limitation of this study particularly, when the subjects were further stratified according to their ethnic origins, such as Malaysian Chinese, Indian and Malay (Table 4). This is because when a small sample size is used, there is a high possibility that the observations observed would be due to chance hence, the estimates stemming from the study may be unintentionally biased. Given this, the data of the present study need to be carefully interpreted. Nevertheless, the number of recruits in each ethnic-based sub-group reflects the racial distribution of patients visiting the UMMC eye clinic.

Secondly, since the prevalence of AMD varies substantially between ethnicity/population, the inclusion of subjects from diversified ethnic origins in the initial analysis may have impeded the true reflection of the disease risk and variants of the genes examined. Nonetheless, we believe that this study has value as an investigation of AMD genotype–phenotype correlation and that these data can contribute to the body of knowledge as well as serve as a starting point for future prospective studies evaluating such associations in a larger multi-ethnic Malaysian population.

Thirdly, is the lack of study's focus on the clinical outcomes in AMD (e.g., types, severity). This is because the study's primary objective was to explore the genetic associations and genotype–phenotype correlations. However, indeed, this omission restricts the comprehensive understanding of how AMD manifests within various ethnic groups. Understanding the clinical nuances, including types and severity, is pivotal as it could offer crucial insights into tailored treatment approaches and disease management strategies specific to each ethnicity. Acknowledging this limitation underscores the necessity for future research to encompass not only genetic associations but also the diverse clinical presentations of AMD across ethnic populations for a more holistic understanding and improved patient care.

Conclusion

In summary, we have found significant associations between SNPs at *HTRA1* and *ARMS2* genes and AMD in the Malaysian population. However, there was no association of AMD with *CFH* gene polymorphism was found. These findings suggest that the sequence of pathways by which AMD develops in the Malaysian population may differ from those present in European populations but are consistent with our Asian counterparts. Nevertheless, the AMD genotype–phenotype correlations observed in this study need to be further validated in a clinically representative multi-ethnic Malaysian population particularly, by considering other genetic risk alleles associated with AMD development as well as confounding factors including diet (e.g., vitamins C and E, beta carotene, and zinc supplements), lifestyle (e.g., smoking status), epigenetics, environmental conditions, and the presence of other co-morbidities.

LIST OF ABBREVIATIONS

AMD	Age-related macular degeneration
AREDS	Age-related eye disease study
ARMS2	*Age-Related Maculopathy Susceptibility 2* Gene
CFH	*Complement Factor H* Gene
CI	Confidence intervals
HTRA1	*Human High-Temperature Requirement A-1* Gene
HWE	Hardy–Weinberg equilibrium
OR	Odd ratios
PCR	Polymerase chain reaction
SD	Standard deviation
SNP	Single-nucleotide polymorphism
Tyr	Tyrosine
UMMC	Universiti Malaya Medical Centre
X^2	Chi-square test

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Author contributions

FI: Conceptualization, Methodology, Formal analysis, Visualization, Investigation, Writing—Original Draft, Writing—Review & Editing, Approved the final draft. SMJ: Conceptualization, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition, Approved the final draft. LCC: Methodology, Writing—Original Draft, Approved the final draft. JJJ: Formal analysis, Visualization, Writing—Original Draft, Writing—Review & Editing, Visualization, Approved the final draft. VS: Conceptualization, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition, Approved the final draft.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the UMMC Ethical Committee (Institutional Review Board) following the ICH-GCP guideline and the Declaration of Helsinki (Reference No.: 757.48). Written informed consent was obtained from patients before they were enrolled on this study.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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