



Original research

The effect of complement factor B gene variation on age-related macular degeneration in Iranian patients

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Abstract

Purpose: To determine the possible association of rs4151667 (L9H) complement factor B (CFB) gene with age-related macular degeneration (AMD). The L9H is one of the functional variations of the CFB. CFB gene encodes the most important protein of the complement system.

Methods: Two hundred sixty-six patients with AMD and 194 unrelated age/sex-matched controls were genotyped for CFB gene (rs4151667) using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. All research subjects were selected from three regions of Iran (Tehran, Tabriz, and Gonabad).

Results: The results showed a significant difference between the frequency of non-TT genotype in total patients and controls [odds ratio (OR) = 0.424, $P = 0.038$]. The analysis for each studied region showed that in patients originating from the Gonabad population, the frequency of TT and non-TT genotypes between patients and the control group were significantly different (OR = 2.894, $P = 0.046$ for TT genotype and OR = 0.346, $P = 0.026$ for non-TT genotype). In patients originating from Tabriz population, TT and non-TT genotypes and A allele revealed considerably different frequencies between the patient and control groups (OR = 3.043, $P = 0.017$; OR = 0.329, $P = 0.013$ and OR = 0.347, $P = 0.048$, respectively). Analysis of patients from Tehran also showed that there was a significant difference in the frequency of TT genotype between patients and controls (OR = 2.168, $P = 0.04$).

Conclusions: The results of the current study indicated a possible protective role for non-TT genotype in L9H variation CFB gene against AMD in a sample of the Iranian population. The region segregation results showed that TT genotype might be a risk factor for susceptibility to AMD. Copyright © 2019, Iranian Society of Ophthalmology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Age-related macular degeneration; Complement factor B; Variation; PCR-RFLP

Introduction

Age-related macular degeneration (AMD) is one of the most common irreversible causes of vision loss in the elderly

population.¹ Optical disturbance in AMD is associated with the degeneration of retinal pigment epithelium (RPE) cells, photoreceptor cells, Bruch's membrane, and the choroidal circulation. Damage of these cells results in chronic inflammation in the eye, and the extracellular formation of these cells leads to an abnormal deposition called drusen.² Advanced AMD is clinically divided into two types: dry (geographic atrophy) and wet (choroidal neovascularization). The dry type is distinguished by progressive atrophy in RPE and photoreceptor cells. In contrast, the wet type arises by the

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abnormal growth of new vessels in the retina, which may lead to the sudden loss of vision.³

The evidence suggests that AMD is a complex disease, and several effective factors including aging, smoking, obesity, hypertension, race, UV exposure, and genetic factors have been reported.⁴ The complement system plays roles as defending against pathogens and providing immunity by adaptive immune responses, destruction of immune complexes, and apoptotic cells.⁵ Several studies have demonstrated that C3, C5, complement factor B (CFB), CFI, and CFH genes of the complement system are expressed in human RPE cells.^{6,7} The analyzing drusen structures, derived from RPE and choroidal vasculature, indicated a wide range of components related to the complement system.⁸ CFB, a serine protease, is an essential protein regulating the complement cascades during which this protein is broken down into two subunits Ba and Bb by factor D. The subunit precursor Bb along with C3b -as convertase-form the alternative pathway.^{9,10} CFB protein is produced in several parts of the eye including the choroid, RPE, and neural retina.¹¹ Previous studies have shown that pro-inflammatory cytokines of TNF- α and IFN- γ can up-regulate the expression of CFB in RPE cells.^{12,13} A study performed by Crowley et al. in a mouse model showed that injection of TLR4 ligand lipopolysaccharide (LPS) enhanced the ocular production of CFB and C3 proteins. They also observed that in LPS-induced CFB and C3 knockout mice, the amounts of active complement proteins were remarkably diminished. Therefore, this indicates the important function of C3 and CFB in the complement system activation.¹⁴ The rs4151667 T > A is located in exon 1 of the CFB gene (chromosome 6) which leads to the exchange of leucine with histidine at position 9. This region of protein may play a role as secretion signal peptide. The presence of the minor allele (A allele) in the genotypes reduces the activity of the complement system and therefore has a probable protective impact against the AMD disease.⁸

Several studies in the USA,^{8,15} the Netherlands,^{16,17} Spain,¹⁸ and China¹⁹ have shown significant association between L9H CFB with AMD. The purpose of this study was to investigate the possible association of CFB gene variation (L9H) in the susceptibility to AMD among Iranian patients.

Methods

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Ophthalmic Research Center, Tehran University of Medical Sciences, Tehran, Iran and the Ethical Board of Gonabad University of Medical Sciences, Gonabad, Iran. All subjects submitted informed consent. This study included 266 patients (158 male and 108 female; 224 wet AMD and 42 dry AMD) with advanced AMD and 194 (106 male and 88 female) healthy, unrelated, age- and sex-matched controls. All AMD patients and controls were referred from hospitals in Tabriz, Tehran, and Gonabad. The exclusion criteria were as follow: retinal diseases other than AMD such as high myopia, retinal dystrophies, central serious retinopathy, vein occlusion,

diabetic retinopathy, uveitis, and systemic inflammatory disease. The controls constituting the study included those that were of age 50 years or older with absence of diagnostic criteria for AMD (individuals with no drusen or RPE changes) and absence of other retinal abnormality or systemic inflammatory disease. All participants underwent a standard ophthalmic exam, including measurement of vision acuity, Slit-lamp examination, and fundoscopy through a dilated pupil. Furthermore, fluorescein angiography, optical coherence tomography (OCT) (an optical analog of ultrasound imaging that uses low coherence interferometry to produce cross-sectional images of the retina), and indocyanine green angiography (ICG) (on patients with wet type and those suspected to be wet type) was performed, and diagnosis of AMD was confirmed according to the international age-related maculopathy (ARM) Epidemiological Study Group criteria.²⁰ AMD was defined by geographic atrophy or choroidal neovascularization in at least one eye. Cases were classified according to the eye with the most severe disease. In the control subjects, no signs of macular pathology or early AMD such as drusen or irregular pigmentations of RPE in the macular area were ophthalmoscopically observed. The control subjects who referred to the general clinics for non-ocular problems were selected after examination by the retina specialists.

After taking blood samples from all individuals, genomic DNA was extracted as described previously.²¹ All 460 DNA samples were successfully genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique. The primer sequences were forward: 5'-AGTGATGTGGGTAGGACAGG-3' and reverse: 5'-TTGGA-GAAGTCGGAAGGAG-3'. The amplified CFB fragments containing a polymorphic site were digested by restriction enzyme Bts α I (Thermo Scientific, USA). The product of PCR is 456 base pairs (bp) that the enzyme can produce two pieces of 234 bp and 222 bp in the presence of A nucleotide, but in the presence of T nucleotide, PCR product is left uncut. To confirm the allelic accuracy of PCR products, some samples were randomly selected for direct sequencing. The frequency distribution of different genotypes was evaluated by Hardy-Weinberg equilibrium (HWE). The comparison of alleles and genotypes' frequencies between patients and healthy controls was performed using the Chi-square and Fishers exact test. *P* values < 0.05 were considered statistically significant.

Results

The distribution of genotypes' frequencies of all studied samples was in HWE (data not shown). The number of participants in this study was 460, which includes 266 (59.4% male and 40.6% female) patients and 194 (54.6% male and 45.4% female) healthy controls (*P* = 0.308). The mean \pm standard deviation (SD) age in AMD patients was 74.50 \pm 7.66, and in healthy controls, it was 72.27 \pm 6.43 years (*P* = 0.157). Baseline features of study groups have been summarized in Table 1. The results of genotypic and allelic frequencies and statistical analysis of CFB rs4151667 (L9H) variation is illustrated in Table 2.

In the total population analysis, from 266 AMD patients, 249 patients (93.6%) were in TT genotype and 17 patients (6.4%) in non-TT genotype (AA = 0 and AT = 17). From 194 subjects in the control group, 167 (86.1%) were in TT genotype and 27 (13.9%) in non-TT genotype (AA = 1 and AT = 26). Non-TT genotype was significantly lower in AMD patients than that of total controls in the Iranian population [odds ratio (OR) = 0.424, *P* = 0.038].

In addition to the total analysis, the data of three regions of Iran (Gonabad in the northeast, Tabriz in the northwest, and the capital Tehran) were analyzed independently. In patients originating from Gonabad, there was a significant association of TT genotype (95.5% vs. 88%, OR = 2.894, *P* = 0.046) with AMD and non-TT genotype (4.5% vs. 12%, OR = 0.346 and

P = 0.026) with resistance to AMD between patients and controls.

Among patients from Tabriz, the frequencies of TT, non-TT genotypes, and A allele were significantly different between patients and controls (94.6% vs. 85.2%, OR = 3.043, *P* = 0.017; 5.4% vs. 14.8%, OR = 0.329, *P* = 0.013; and 2.7% vs. 7.4%, OR = 0.347, *P* = 0.048, respectively).

Analysis of patients from Tehran showed that there was a significant difference between patients and controls in the frequency of TT genotype (92.8% vs. 85.6%, respectively, OR = 2.168, *P* = 0.040).

Discussion

AMD is an epidemic multi-factorial disease with a prevalence of 8.7% in the world's population, and it has been estimated that by the year 2020, the number of affected people would be about 196 million.²² According to a report, this figure for Iran is less than the global average (5.8%).²³ Genome-wide studies have identified multiple genetic loci involved in AMD disease, and these have led to this fact that several important biological pathways, including the complement system, are important in the pathogenesis of the disease.^{24,25} There is a report showing the increase of CFB in the

Table 1
Baseline features of subjects.

	AMD (266)		Control (194)		P*
Age	Mean ± SD 74.5 ± 7.66		72.27 ± 6.43		0.157
Sex	Male	Female	Male	Female	
	59.4% (158)	40.6% (108)	54.6% (106)	45.4% (88)	0.308

AMD: Age-related macular degeneration; SD: Standard deviation.
*Based on Chi-Square test.

Table 2
Comparison of frequency distribution of genotypic and allelic complement factor B (CFB) gene (rs4151667) between age-related macular degeneration (AMD) and control groups in Iran and the three regions of Iran (Gonabad, Tabriz, and Tehran).

Genotype	Total-AMD n = 266	Total-control n = 194	OR	95% CI		P
				Lower	Upper	
non-TT (AA + AT)	17 (6.4%)	27 (13.9%)	0.424	0.141	1.224	0.038*
TT	249 (93.6%)	167 (86.1%)	2.361	0.817	7.069	0.052
Allele						
A	18 (3.4%)	27 (7%)	0.468	0.101	1.980	0.139
T	514 (96.6%)	361 (93%)	2.139	0.505	9.933	0.210
Genotype	Gonabad-AMD n = 44	Gonabad-control n = 50	OR	95% CI		P
				Lower	Upper	
non-TT (AA + AT)	2 (4.5%)	6 (12%)	0.346	0.096	1.158	0.026*
TT	42 (95.5%)	44 (88%)	2.894	0.863	10.397	0.046*
Allele						
A	2 (2.3%)	6 (6%)	0.369	0.057	1.947	0.121
T	86 (97.7%)	94 (94%)	2.711	0.514	17.449	0.181
Genotype	Tabriz-AMD n = 56	Tabriz-control n = 54	OR	95% CI		P
				Lower	Upper	
non-TT (AA + AT)	3 (5.4%)	8 (14.8%)	0.329	0.103	0.995	0.013*
TT	53 (94.6%)	46 (85.2%)	3.043	1.005	9.710	0.017*
Allele						
A	3 (2.7%)	8 (7.4%)	0.347	0.064	1.603	0.048*
T	109 (97.3%)	100 (92.6%)	2.880	0.624	15.559	0.056
Genotype	Tehran-AMD n = 166	Tehran-control n = 90	OR	95% CI		P
				Lower	Upper	
non-TT (AA + AT)	12 (7.2%)	13 (14.4%)	0.461	0.162	1.278	0.059
TT	154 (92.8%)	77 (85.6%)	2.168	0.782	6.177	0.040*
w						
A	13 (3.9%)	13 (7.2%)	0.523	0.123	2.081	0.102
T	319 (96.1%)	167 (92.8%)	1.912	0.481	8.139	0.114

AMD: Age-related macular degeneration; CFB: Complement B-Factor gene; OR: Odds ratio; CI: Confidence interval. *: Statistically significant

Table 3

Association results of complement factor B (CFB) gene polymorphism with age-related macular degeneration (AMD) in other studied populations.

First author	Country	Ethnicity	Allele% (Case/control)		Significance in disease
			Minor	Major	
Gold B, 2006 ⁸	USA	Caucasian	2/5.6	98/94.4	Protective effect of A allele ($P = 3.93 \times 10^{-6}$). Haplotype containing C2 E318D/CBF L9H pair was also significantly protective ($P = 3.4 \times 10^{-6}$).
Maller J, 2006 ¹⁵	USA	Caucasian	2.9/5	97.1/95	Protective effect of A allele ($P = 0.0002$).
Jakobsdottir J, 2008 ³⁵	USA	Caucasian	2.8/3.6	97.2/96.4	Non-significant association.
Park K, 2009 ³⁶	USA	Caucasian	2.5/4.7	97.5/95.3	Non-significant association. No interaction between CFB, L9H; C2, rs547154.
Bergeron-Sawitzke J, 2009 ³⁷	USA	Caucasian	2/5.1	98/94.9	TT as risk factor ($P = 0.005$). Haplotype association (rs9332739, rs547154, rs4151667, rs4151659, and rs2072633)
Lima LH, 2010 ³⁸	USA	Caucasian	2/4.3	98/95.7	Significant A allele ($P = 0.002$). Tendency towards correlation ($P < 0.2$).
Smailhodzic D, 2012 ¹⁶	The Netherlands	Caucasian	6.1/8	93.9/92	Protective effect of AT genotype ($P = 0.042$). Increased CFB protein in AMD.
Van de Ven JPH, 2012 ¹⁷	The Netherlands	Caucasian	2.8/4.9	97.2/95.1	A allele was associated in AMD with cuticular drusen ($P = 0.027$) and in non-cuticular drusen AMD (0.006).
Caire J, 2014 ¹⁸	Spain	Caucasian	1.9/3.9	98.1/96.1	Protective effect of A Allele ($P = 0.009$). Association with rate of progression ($P = 0.04$).
Buentello-Volante B, 2012 ³⁹	Mexico	Caucasian	1.6/1.3	98.4/98.7	Non-significant association.
Contreras AV, 2014 ⁴⁰	Mexico	Caucasian	0.7/1.7	99.3/98.3	Non-significant association.
McKay GJ, 2009 ⁴¹	the UK	Caucasian	3.3/5.1	96.7/94.9	Non-significant association.
Farwick A, 2009 ⁴²	Germany	Caucasian	2.4/3.4	97.6/96.6	Non-significant association.
Richardson AJ, 2008 ⁴³	Australia	Caucasian	2.2/3.7	97.8/96.3	Non-significant association.
Kaur I, 2010 ⁴⁴	India	Asian	4/6.3	96/93.7	Non-significant association. Strong linkage disequilibrium
Kim S J, 2012 ⁴⁵	Korea	Asian	0.7/1.8	99.3/98.2	Non-significant in AMD and smoking status.
Pei XT, 2009 ⁴⁶	China	Asian	2/3.1	98/96.9	Non-significant association. Non-interaction between C3 rs2250656–CFB rs4151667.
Liu X, 2010 ⁴⁷	China	Asian	1.7/1.6	98.3/98.4	Non-significant association.
Lu F, 2013 ¹⁹	China	Asian	1.1/2.7	98.9/97.3	A allele was associate ($P = 0.0094$)
Wu L, 2013 ⁴⁸	China	Asian	3.9/2.3	96.1/97.7	Non-significant association. Haplotype of rs9332739–rs4151667 are significant (G-A vs. G-T, $P = 0.0058$).
Tanaka K, 2014 ⁴⁹	Japan	Asian	1.8/2.4	98.2/97.6	Non-significant association in allele and genotype. Haplotypes of TAT (rs541862-rs2072633-rs4151667) and CGT showed strong associations in the polypoidal CNV, typical PCV and control.

AMD: Age-related macular degeneration; CFB: Complement B-Factor gene; PCV: Polypoidal choroidal vasculopathy; CNV: Choroidal neovascularization; CGT: Capital gains tax.

retinal drusen and Bruch's membrane of AMD patients.⁸ In addition, increased concentrations of C3d and Ba fraction of CFB are observed in the blood samples from AMD patients. The presence of active compounds in the blood circulation indicates that AMD inflammation is not only limited to the retina but is also systemic.²⁶ The investigation of Reynolds et al. has shown that the complement system activity plays an important role in the formation of drusen by disturbing the extracellular matrix and the secreted structures of the retinal cells.²⁷ These published data confirm the important role of the complement system in the development of AMD.

So far, several genes involved in the complement system such as C3, CFI, and CFH have been studied in samples of the Iranian population in which C3 (rs2230199), CFI (rs141853578), and CFH (rs2274700, rs3753395, rs800292, and rs1061170) genes have shown significant association with AMD in this population.^{28–34} Several studies have been conducted on the association of CFB gene variation and AMD. A

summary of relevant results is given in Table 3. The previous studies in different populations have shown different results. For instance, the results of Gold et al.⁸ and Maller et al.'s¹⁵ studies in the USA showed rs4151667 variation has a protective effect in AMD, but others showed no association.^{35,36} Studies in the Netherlands^{16,17} and Spain¹⁸ illustrated a protective effect of A allele in AMD. The studies in Mexico,^{39,40} the UK,⁴¹ Germany,⁴² Australia,⁴³ and Korea⁴⁵ declared no association of this variation with AMD. The study of Liu et al.⁴⁷ showed no association of CFB gene rs4151667 variation with AMD in the Han Chinese population. However, another study conducted by Lu et al. on the same population has reported a significant association between rs4151667 variation and AMD.¹⁹ In a study conducted on the Indian population, there was no association between rs4151667 variation and AMD despite the observation of linkage disequilibrium.⁴⁴ Therefore, the effects of CFB variation on AMD in white populations are more important than in the

Asian population.⁵⁰ Different results in the study of the association of CFB gene polymorphisms with AMD in different populations could be due to differences in genetic backgrounds among populations.

The majority of Iran's population is ethnically Persian, although various ethnicities also reside in Iran. The dominant population of Tehran (the capital) is Persian. Turkish-Azeri population of Tabriz (in the northwest) is genetically close to the Caucasian race and is the second ethnic majority of Iran. Due to the existence of specific traditions in Gonabad (in the northeast), its people have a unique and isolated gene pool.^{51,52} Therefore, AMD patients from these three different regions were selected for the genetic studies. In our study, CFB gene variation and its association with AMD in three different regions of Iran were analyzed altogether as well as separately. The conducting analysis on these three regions showed significant associations in each region. Analyzing data from the Tehran population showed a significant association of TT genotype with AMD disease. In patients originating from Tabriz, there were significant differences in the frequencies of TT and non-TT genotypes and A allele between AMD and those of the control groups. Individuals from this population carrying TT genotype have a 3.043 times greater risk of developing AMD. In addition, we found significant differences in frequencies of non-TT and TT genotypes between AMD and control groups in the Gonabad population. When we pooled all three regions' data, the total analysis showed an association of non-TT genotype with resistance to AMD in Iranian patients.

Observing different results in terms of CFB gene association with AMD disease in different populations could be due to differences in the genetic backgrounds of these populations. AMD is a complex disease which could be an outcome of the interactions of several genetic factors with each other and with non-genetic factors.⁴ Therefore, it is acceptable to see different contributions of each gene in the development of the disease in different populations.

Sample size and possible enrollment of other ethnicities in the samples are considered limitations. It is recommended to classify AMD patients into several subgroups, a group of patients with high exposure to light, a smoking group, and also classification on the base of their gender and age in order to study the effect of these important factors in the development of the disease.

In conclusion, in this study, we analyzed the data of three areas separately and achieved significant results in rs4151667 (L9H) variation of CFB gene. CFB gene has a possible role in the prevalence of AMD in the northeastern and northwestern parts of the country as well as the capital. Non-TT genotype plays a relatively protective role against AMD in the total population, and TT genotype is a susceptible factor of AMD in three demographic regions of Iran.

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