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ORIGINAL PAPER

Association of polymorphisms of complement factor I rs141853578 (G119R) with age-related macular degeneration in Iranian population

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Abstract

Background Age-related macular degeneration (AMD) is a complex disease, and recent studies have shown role of complement system genes in its development. Complement factor I regulates the complement pathways, and relationship between CFI polymorphisms and AMD is controversial. We evaluated the possible association of complement factor I rs141853578 (G119R) variation with advanced AMD in Iranian patients.

Materials and methods We included 371 case–control samples consisting of 220 advanced AMD patients and 151 genetically unrelated healthy controls. Extracted DNA samples amplified to obtain fragment including the polymorphic complement factor I rs141853578 (G119R) region.

Results The distribution of the genotypes was significantly different in the AMD patients compared to that of controls ($p = 0.035$). The TT genotype frequencies for CFI were significantly higher in AMD group (7.7 vs. 2%, OR 4.67, CI 1.33–16.45, $p = 0.016$). This significant difference was maintained after adjustment for the effects of age and gender (OR 5.09, CI 1.42–18.20, $p = 0.012$). The minor allele frequency (T allele) was also significantly higher in AMD patients compared to that of controls (29.3 vs. 21.5% OR 1.51, CI 1.07–2.13, $p = 0.018$).

Conclusion Current study showed that CFI rs141853578 (G119R) is a risk factor for developing advanced type AMD. This study also suggests that the frequency of G119R polymorphism in our population is not as rare as reported from other populations.

Keywords Age-related macular degeneration (AMD) · Complement factor I (CFI) rs141853578 (G119R) · Single nucleotide polymorphism

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Introduction

Role of genetic components in age-related macular degeneration (AMD) has been shown in recent years [1–3]. Increasing prevalence of AMD and its deleterious effect on the life quality highlight the importance of its etiologic components. Strong evidences have shown important role of inflammatory and complement system-related genes in AMD [4–7]. These findings implicate pivotal role of the complement

system in the susceptibility to this disease. Complement component 3 (C3) is the central element of the complement cascade, and its genetic polymorphisms have shown to be involved strongly in AMD [1, 4, 7]. Complement factor I (CFI), a serum protease, regulates the complement pathways by inactivating C3 [8]. Altered function of CFI in AMD has previously been shown [9]; however, the association of CFI polymorphisms with AMD is controversial. Yang et al. [10] reported that none of the studied CFI gene polymorphisms (rs10033900 or rs2285714) was a risk factor for neovascular AMD in a Chinese population. Association study on AMD patients from UK has also shown no association of rs10033900 with the disease [11]. However, Fagerness et al. [12] showed that the single nucleotide polymorphism (SNP) of rs10033900 near the CFI gene is associated with AMD. This risk effect of CFI gene in AMD was replicated in other studies in different populations [13–18]. Recently, a rare missense mutation, rs141853578 (Gly119Arg), has been shown to confer a high risk of AMD via the disturbed regulation of C3b degradation [17, 18]. This suggested SNP as an AMD risk variant did not reach statistical significance in other studies [19, 20]. Although several studies from different population have suggested the implication of CFI gene variants in AMD development, CFI rs141853578 (G119R) locus polymorphism has not widely been studied in different population. In this study, we planned to evaluate the possible association of CFI variant, rs141853578 (G119R), with advanced AMD in our cohort.

Materials and methods

Study population

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and Ethical Board of Gonabad University of Medical sciences, Gonabad, Iran. The written informed consent was obtained from all participants for the study and use of blood and DNA for AMD-related research projects. This study consisted of Iranian AMD cases and healthy control subjects. Individuals with AMD in at least one eye were recruited from Labbafinejad Medical Center, Tehran; 22th Bahman Hospital, Gonabad and Nikukari Eye Hospital, Tabriz. We included 371 case–control samples consisting of 220 advanced type AMD patients and 151 genetically unrelated healthy controls according to inclusion and exclusion criteria described below. Cases and controls included after standard ophthalmologic examination by a retinal specialist.

Inclusion and exclusion criteria

Patients with the diagnosis of AMD and unrelated healthy individuals aging older than 50 years included in this study. The exclusion criteria included the 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 the absence of diagnostic criteria for AMD (individuals with no drusen or RPE changes) and the absence of other retinal abnormality or systemic inflammatory disease.

Diagnosis of AMD

All participants underwent a standard ophthalmic exam, including measurement of vision acuity, Slit-Lamp examination and fundoscopy through a dilated pupil. Further, fluorescein angiography, indocyanine green angiography (ICG) and optical coherence tomography (OCT) were performed and diagnosis of AMD was confirmed. 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 the RPE in the macular area were ophthalmoscopically observed.

Genotyping

Genomic DNA was extracted from peripheral white blood cells of whole-blood samples using standard laboratory protocols. Extracted DNA samples amplified to obtain fragment including the polymorphic region rs141853578 (G119R), by using primers: Forward: CTCCAGCTGCTTTTGCATATGA and Reverse: TGATGTTCAAAGCTCACTTGACA. The PCR was performed in a DNA thermocycler, and

Table 1 Baseline features of study groups

		Total	Control	AMD			P1	P2	P3	P4
				Total	Wet	Dry				
Age	Mean ± SD	74 ± 7	72 ± 6	75 ± 8	75 ± 8	76 ± 8	< 0.001†	< 0.001†	0.022†	0.597†
Sex	Male	225 (60.6%)	86 (57.0%)	139 (63.2%)	132 (65.0%)	7 (41.2%)	0.228*	0.123*	0.215*	0.050*
	Female	146 (39.4%)	65 (43.0%)	81 (36.8%)	71 (35.0%)	10 (58.8%)				

P1 comparison of AMD versus control, *P2* comparison of wet AMD versus control, *P3* comparison of dry AMD versus Control, *P4* comparison of wet versus dry AMD

†Based on *t* test

*Based on Chi-square test

samples were denatured at 94 °C for 5 min followed by 35 cycles under the following conditions: denaturing at 94 °C for 30 s, annealing at 58 °C and extension at 72 °C for 45 s. The final extension step was lengthened to 5 min. In order to determine the genotypes of individuals, PCR product (221 bp) was subjected to restriction enzyme digestion using *N1aIII* at 37 °C for overnight. In the presence of T allele, PCR product was left uncut. However, in the presence of C allele the fragment was cut into 86 and 135 bp. The genotypes of several randomly selected samples were reconfirmed by direct sequencing.

Statistical analysis

All statistical analysis performed by R (R Core Team 2014, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org). To assess the normal distribution of data, we used Kolmogorov–Smirnov test and *Q–Q* plot. To present data, we used mean, standard deviation, median and range, frequency and percent. To evaluate the differences between groups, we used *t* test, Chi-squared and Fisher exact test. To evaluate the odds ratio and adjusted odds ratio, we used logistic regression. *p* value less than 0.05 considered statistically significant.

Results

We included 371 case–control samples consisting of 220 AMD patients (63.2% male, including 203 wet AMD and 17 advanced dry AMD) and 151 genetically unrelated healthy controls (57% male). The mean ± SD age in AMD group was 75 ± 8 compared to control group which was 72 ± 6 ($p < 0.001$, Table 1).

We confirmed that the genotypic frequencies in both AMD patients and controls were consistent with Hardy–Weinberg equilibrium (data not shown). Baseline features of study groups are summarized in Table 1. Genotype and allele frequencies of the polymorphisms of the CFI rs141853578 (G119R) are listed in Table 2.

The distribution of the genotypes was significantly different in the AMD patients compared to that of controls ($p = 0.035$). The frequency of TT genotype for CFI was significantly higher in AMD group (7.7 vs. 2%, OR 4.67, CI 1.33–16.45, $p = 0.016$). This significant difference was maintained after adjustment for the effects of age and gender (adjusted OR 5.09, CI 1.42–18.20, $p = 0.012$). This statistically significant association of TT genotype with AMD disease was also observed in wet type of AMD disease (Table 2).

The allele frequency for CFI rs141853578 (G119R) was found to be significantly different between AMD and normal controls even after adjustment of for age and sex factor (Table 2). The minor allele frequency (T allele) was significantly higher in AMD patients than that of controls (29.3 vs. 21.5% OR 1.51, CI 1.07–2.13, $p = 0.018$).

Discussion

Present study performed on Iranian patients confirmed Alexander et al. [17] and van de Ven et al. [18] reports showing risk effect of CFI rs141853578

Table 2 Genotype and allele distribution among AMD patients and control group

		Control	AMD	OR	95% CI		*p*	AOR	95% CI		*p*
					Lower	Upper			Lower	Upper	
Genotype[¤]							0.036				0.035
	CC	89 (58.9%)	108 (49.1%)	Ref				Ref			
	CT	59 (39.1%)	95 (43.2%)	1.33	0.86	2.04	0.197	1.28	0.81	2.03	0.296
	TT	3 (2.0%)	17 (7.7%)	4.67	1.33	16.45	0.016	5.09	1.42	18.20	0.012
Allele[§]											
	C	237 (78.5%)	311 (70.7%)	Ref				Ref			
	T	65 (21.5%)	129 (29.3%)	1.51	1.07	2.13	0.018	1.55	1.07	2.22	0.019
		Control	Wet	OR	95% CI		*p*	AOR	95% CI		*p*
					Lower	Upper			Lower	Upper	
Genotype[¤]							0.041				0.036
	CC	89 (58.9%)	103 (50.7%)	Ref				Ref			
	CT	59 (39.1%)	83 (40.9%)	1.22	0.78	1.88	0.382	1.12	0.70	1.80	0.636
	TT	3 (2.0%)	17 (8.4%)	4.90	1.39	17.26	0.013	5.34	1.49	19.12	0.010
Allele[§]											
	C	237 (78.5%)	289 (71.2%)	Ref				Ref			
	T	65 (21.5%)	117 (28.8%)	1.48	1.04	2.09	0.029	1.49	1.03	2.16	0.036
		Control	Dry	OR	95% CI		*p*	AOR	95% CI		*p*
					Lower	Upper			Lower	Upper	
Genotype[¤]							0.070				0.088
	CC	89 (58.9%)	5 (29.4%)	Ref				Ref			
	CT	59 (39.1%)	12 (70.6%)	3.62	1.21	10.81	0.021	3.52	1.15	10.81	0.028
	TT	3 (2.0%)	0 (0.0%)	NA				NA			
Allele[§]											
	C	237 (78.5%)	22 (64.7%)	Ref				Ref			
	T	65 (21.5%)	12 (35.3%)	1.99	0.93	4.23	0.074	1.88	0.86	4.09	0.112
		Dry	Wet	OR	95% CI		*p*	AOR	95% CI		*p*
					Lower	Upper			Lower	Upper	
Genotype[¤]							0.142				0.094
	CC	5 (29.4%)	103 (50.7%)	Ref				Ref			
	CT	12 (70.6%)	83 (40.9%)	0.34	0.11	0.99	0.048	0.29	0.10	0.88	0.030
	TT	0 (0.0%)	17 (8.4%)	NA				NA			
Allele[§]											
	C	22 (64.7%)	289 (71.2%)	Ref				Ref			
	T	12 (35.3%)	117 (28.8%)	0.74	0.36	1.55	0.427	0.73	0.35	1.56	0.419

OR odds ratio, *AOR* adjusted odds ratio, consider the effect of age and sex

[¤]Based on logistic regression

[§]Based on GLMM analysis

(G119R) SNP in advanced type AMD. CFI as a serum protease regulates the complement pathways, by inactivating C3 [8]. Although the association of CFI polymorphisms with AMD development is controversial in different populations, recently a meta-analysis has suggested that CFI rs10033900 and rs2285714

polymorphisms may contribute to AMD [21]. CFI G119R has been shown to contribute to the development of AMD by regulation of C3b degradation [17, 18]. van de Ven et al. [18] reported CFI G119R, located at chromosome 4q25, as a rare, highly penetrant missense mutation which confers a high risk of AMD due to altered C3b degradation. Alexander et al. [17] confirmed the findings of van de Ven et al. that the G119R substitution confers a high risk of AMD. They, however, suggested that this missense mutation is not as rare as van de Ven et al. reported. On the other hand, this suggested SNP as AMD risk variant did not reach statistical significance in other studies [19, 20]. Although Kavanagh et al. [20] did not find statistically significant difference between CFI G119R variants in their study, all carriers of the rare variant were AMD cases (four patients) and all had low serum CFI levels, consistent with possible association of this rare variant with AMD.

Our study reconfirmed risk effect of CFI G119R SNP in advanced type AMD and suggested that this missense mutation is not as rare in our studied patients as previously reported [18]. This difference between our results and above-mentioned studies could be due to the ethnic differences. Genetic studies may pave the road for future personalized treatments. The MAHALO trial suggested that CFI genetic biomarker variability is important for predicting lampalizumab (complement factor D inhibitor) treatment response in advanced dry-type AMD [22]. Our findings support significance of studying CFI genetic variable sites in AMD for different population. Although CFI G119R polymorphism has not been clarified extensively in different population yet, in this study we showed risk effect of CFI G119R SNP in advanced type AMD among Iranian patients.

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Compliance with ethical standards

Conflict of interest No conflict of interest to be declared.

Ethical approval This study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and Ethical Board of Gonabad University of Medical sciences, Gonabad, Iran. The written informed consent was obtained from all participants for the study and use of blood and DNA for AMD-related research projects.

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