Original Article

Association of *CFI* gene polymorphism with age related macular degeneration in Northwest of Iran

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Abstract

Background & Aims: To investigate the association of *CFI* p.Gly119Arg polymorphism with Age-related macular degeneration (AMD).

Materials & Methods: In this case-control study, the association of p.Gly119Arg polymorphism in *CFI* gene was investigated in 65 patients suffering from AMD and150 healthy age, sex and ethnicity matched unrelated people as control group. Both of the case and control groups were originated from Northwest of Iran. Genotypes of both groups were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: Investigation of the association of p.Gly119Arg polymorphism in *CFI* gene with AMD showed that there are no statistically significant differences between patients and controls in genotype and allele frequencies of this polymorphism (P>0/05).

Conclusion: Our result show that p.Gly119Arg polymorphism of the *CFI* gene is unrelated to the susceptibility to AMD in Northwest of Iran.

Keywords: AMD, polymorphism, *CFI* gene

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Introduction

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in people older than 65 years around the world (1). AMD is a degenerative progressive disease that clinically causes a range of phenotypic and pathological symptoms and firstly affects retinal macular (2). In this disease, progressive degeneration of epithelium of retinal pigment (RPE) causes impaired vision of patients (3).

The main characteristic of the early stages of the diseases is a yellow extracellular deposit known as Drusen between the retinal pigment epithelium and brook membrane (4). Drusen can be classified both as hard Drusen(small discrete nodes) and soft Drusen (large nodes with unknown boundary) (5,6). Software Drusen results in extensive damage to the retina, retinal pigment epithelium and choroid and ultimately leads to advanced AMD (CNV, GA) (7). Geographic atrophy in dry AMD occurs along with degenerative changes in

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pigment epithelium membrane and photoreceptors (8).In contrast, wet AMD can be identified with Choroidal angiogenesis, wound and bleeding under the retina and severely weakens the patient's central vision and ultimately causes blindness (9).

AMD is a multi_factorial complex disease and ageing. Obesity and a sedentary lifestyle, high blood pressure, tobacco and genetics are the leading factors for this disease (10- 12). The complement system is responsible for the defense against pathogens, the adaptive immune response, and also destruction of immune complexes and apoptotic cells (13). A number of studies on patients with AMD showed the presence of regulatory components and proteins of the complement system in the retinal pigment epithelium (RPE) near Drusen(14). A number of components of the complement system, including CFH, CFB, C2, C3, CFI are the most important sensitive making alleles in the pathogenesis of the disease (15, 16). *CFI* is a serine protease that breaks c4b and c3b and is an important regulator of the classical and complement pathway (17). Studies have shown that beta-amyloid binds to *CFI* and creates disorder in c3b decomposition by *CFI* (18). RPE cell proliferation in the early stages of AMD showed increased expression of *CFI* protein (19).

Genetic studies in recent years have found that single nucleotide polymorphisms (SNPs) are associated with an increased or reduced risk of AMD (20). Polymorphism of (rs141853578) c.355G> A of *CFI* gene has been shown to have a high permeability at risk of developing AMD while being a rare condition (21, 22). The aim of this study was to investigate the hypothesis that polymorphism of *CFI* gene (rs141853578) may be associated with susceptibility to AMD to in North-West of Iran.

Materials and Methods

215 cases have been studied genetically in this case-control research and placed in polymorphisms (p.Gly119Arg) c.355G>A of *CFI* gene. 65 patients have been suffering from AMD who were referred to Nikookari Eye Hospital in Tabriz and introduced to genetics center by an ophthalmologist. The control group included 150 patients who have no kinship with each other or patients, but with age and gender match with the patient group. Both groups were from the population of the North-west of Iran. Blood samples were collected in both groups after completion of the consent form. Genomic DNA had been extracted from blood using DNA extraction standard protocol (Saturated salt) (23). Polymorphism of *CFI* in c.355G> A were studied using PCR-RFLP method. Primers F: CTCCAGCTGCTTTTGCATATGA and R: TGATGTTCAAAGCTCACTTGACA were used for this polymorphism. PCR Test was performed by denaturation for 5 min at 95 ° and then 34 cycles of amplification (30 sec. at 94 ° C, 30 seconds at 59 ° C, 30 seconds at 72 °) followed by elongation(5 min at 72 ° C) and had a 221 bp product. Restriction enzyme of (NIaIII) Hin1II was used for enzyme digestion which were kept in Water Bath along with buffer for enzyme and PCR products an overnight at 37 ° C. Enzymed PCR digested products were electrophoresis on polyacrylamide gel and were observed in UV light after stained with ethidium bromide. Hin1II enzyme does cut in presence of allele C in the position and created two pieces of 135 and 86 bp. Therefore, 86,135,221 tri-band were detected in samples with CC double bond genotype and samples with CT genotype (Figure 1). Genotypic and allelic differences in the patient and control groups were analyzed using Fisher's exact test and Chi square test. P less than 0.05 is considered significant.

Results

65 patients with AMD and 150 healthy were studied as controls to analyze P.Gly119Arg polymorphisms in *CFI* gene. The age range of patients was 42 to 74 years. 26 of those were women and 39 were men. The genotype

distribution was evaluated between patients and control group. The frequency of genotypes for genotype CC, CT, TT in patients' group were 58.46%, 41.54%, and zero percent, respectively, and in the control group were observed as 59.33%, 38.67%, and 2%. Also allelic frequencies were calculated for both groups and the value for alleles C and T in patients' group were 79.23, and 20.77 and in the control group were 78.66, and 21.34, respectively. Calculated P values for these groups were not significant. The results of the statistical analysis are presented in Table 1.

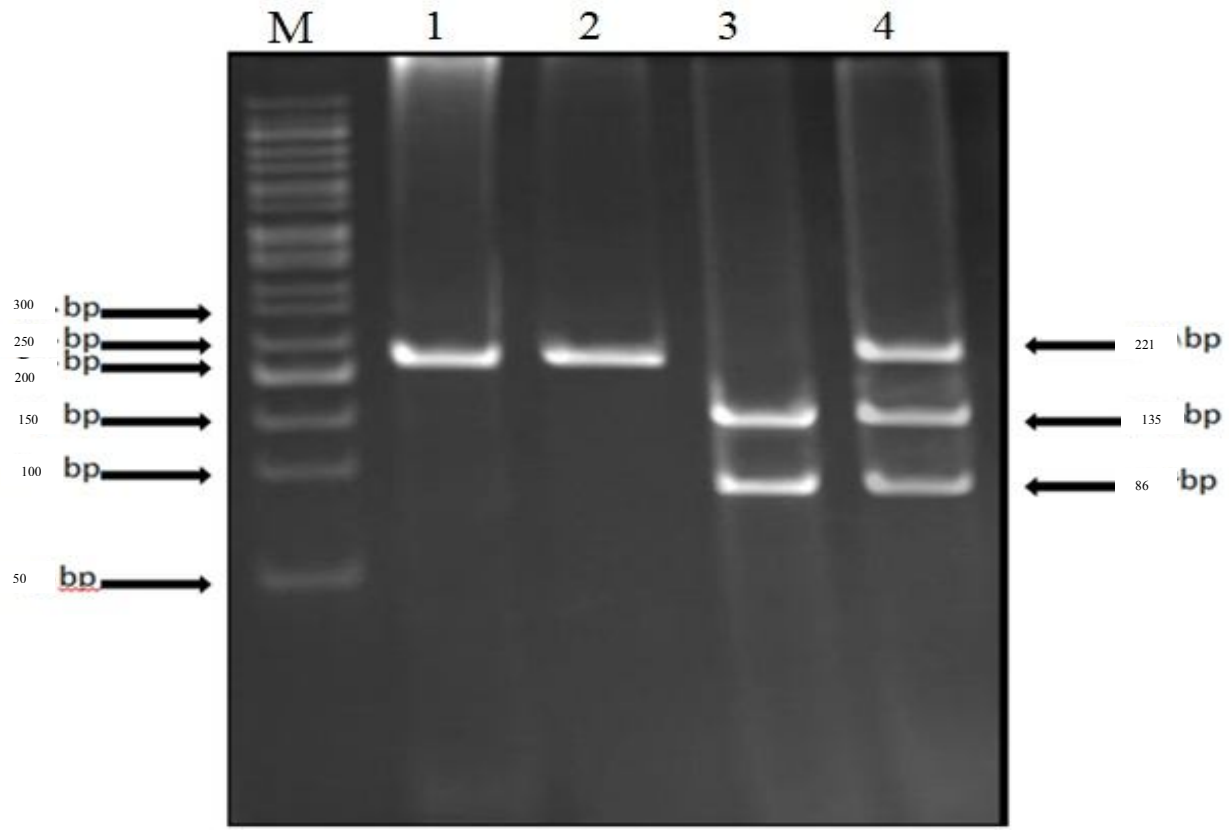

Figure 1- PCR-RFLP data after enzyme digestion by Hin1II on electrophoresis gel for *CFI* gene
M: Marker 1: PCR Product 2: genotype TT 3: genotype CC 4: genotype CT

Table 1. The genotype distribution and bridge allele P.Gly119Arg polymorphism between patients and controls

P Value	Chance Percent	150 Controls		65 Patients		Genotype
		%	Number	%	Number	
0.43	0.96	59.33	89	58.46	38	CC
0.34	1.12	38.67	58	41.54	27	CT
0.24	0.00	2	3	0	0	TT
						Alleles
0.43	1.03	78.66	236	79.23	103	C
0.42	0.96	21.34	64	20.77	27	T

Discussion

Age-related macular degeneration is a common cause of blindness in the elderly people that the main cause of this disease remained unclear. The role of genetics in AMD has been confirmed by the reports of familial aggregation of the disease, similar phenotypes of twins and increased risk of disease in first degree relatives of patients (24). Several studies confirmed the complement proteins (CFH, CFI, C5, C5a, ...) in Drusen (25,26). In addition, the studies have shown that the activity of the complement system plays an important role in the formation of Drusen by disrupting the extracellular matrix and the structure secreted by the cells of the retina (27). These documents confirm the role of the complement pathway in AMD. For example, the association of polymorphisms Y402H in CFH genes with AMD has been observed in several studies as well as HTRA1 gene from ARMS2 family has been reported to have relationship with the disease (24).

Also significant relationship of SNP, placing downstream of *CFI* genes, with AMD have been reported in several GWAS (genome wide association study) (28). For example, the results of. Fagerness JA and colleagues showed that rs10033900 polymorphism in *CFI* gene is associated with AMD in Caucasians patients (29). However, Ennis S and colleagues showed that rs1003390 polymorphism of *CFI* gene is not associated with AMD in UK population (30). In 2013, van de Ven and colleagues showed that as a rare condition, p.Gly119Arg polymorphism in *CFI* gene has very high permeability in the development of AMD (21). The results (p.Gly119Arg involvement in the risk of developing AMD) was confirmed by Philip Alexander et al., pointing out that the mutation is rare and not permeable enough (31). This study showed no significant association between p.Gly119Arg polymorphisms of *CFI* gene and age-related macular degeneration in the North West of Iran. These findings indicate that p.Gly119 Arg polymorphism of *CFI* gene has a direct role in susceptibility to AMD in the population of North West of Iran which is may be due to the involvement of other related mutation in the gene or the role of other genes. However, different results may be achieved through studies in populations with different races or in a larger area.

Conclusion

p.Gly119Arg polymorphism of *CFI* gene was not significantly associated with susceptibility degeneration of age-related macular among population of the North West of Iran. It is recommended, therefore, to conduct studies on the role of these genes in age-related macular degeneration on a broader level as well as investigate the role of polymorphism and other genes that interact with *CFI* gene.

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References

1. Holliday EG, Smith AV, Cornes BK, Buitendijk GH, Jensen RA, Sim X, et al. Insights into the Genetic Architecture of Early Stage Age-Related Macular Degeneration:A Genome-Wide Association Study Meta-Analysis. Plos One 2013;8 (1):e52830.
2. Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv Ophthalmol 1995; 39:367–74.
3. Lu F, Zhao P, Fan Y, Tang S, Hu J, Liu X, et al. An association study of SERPING1 gene and agerelated macular degeneration in a Han Chinese population. Mol Vis 2010;16:1.

4. Thakkinstian A, McKay GJ, McEvoy M, ChakravarthyU, Chakrabarti S, Silvestri G, et al. Systematic review and meta-analysis of the association between complement component 3 and age-related macular degeneration: a HuGE review and meta-analysis. Am J Epidemiol 2011;173(12):1365-79.

5. Bressler NM, Silva JC, Bressler SB, Fine SL, Green WR. Clinic opathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. Retina 1994; 14:130–42.

6. Sarks S, Cherepanoff S, Killingsworth M, Sarks J. Relationship of Basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. Investig Ophthalmol Vis Sci 2007; 48:968–77.

7. Ferris FL, Davis MD, Clemons TE, Lee L-Y, Chew EY, Lindblad AS, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. Arch Ophthalmol 2005;123(11):1570–4.

8. Ratnapriya R CE. Age-related macular degeneration – clinical review and genetics update. Clin Genet 2013;84(2):160-6.

9. Bressler NM. Early Detection and Treatment of Neovascular Age-related Macular Degeneration. J Am Board Fam Pract 2002;15(2):142-52.

10. Meyers SM. A twin study on age related macular degeneration. Trans Am Ophthalmology Soc 1994; 92:775-843.

11. Grizzard SW, Arnett D, and Haag SL. Twin study of age-related macular degeneration. Ophthalalmic Epidemiol 2003; 10:315-22.

12. Dosso AA and Bovet J Monozygotic. twin brothers with age-related macular degeneration. Ophthalmological 1992; 205:24-8.

13. Walport, M.J. Complement First of two parts. N Engl J Med 2001.344, 1058–66.

14. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, et al. Drusen proteome analysis: an approach to the etiology of age related macular degeneration. Proc Natl Acad Sci USA 2002;99:14682–7.

15. 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(5270):384-9.

16. Francis PJ, Hamon SC, Ott J, Weleber RG, Klein ML. Polymorphisms in C2, CFB and C3 are associated with progression to advanced age related macular degeneration associated with visual loss. J Med Gene 2009;46(5):300-7.

17. Nilsson SC, Sim RB, Lea SM, Fremeaux-Bacchi V, Blom AM. Complement factor I in health and disease. Mol Immunol 2011;48(14):1611-20.

18-Wang J, Ohno-Matsui K, Yoshida T, Kojima A, Shimada N, Nakahama K, et al. Altered function of factor I caused by amyloid beta: implication for pathogenesis of age-related macular degeneration. J Immunol 2008; 181:712-20.

19. Kociok, N., Joussen, A.M. Enhanced expression of the complement factor H mRNA in proliferating human RPE cells. Arch Ophthalmol 2010; l(248): 1145–53.

20. Smith C. Genomics: SNPs and human disease. Nature 2005;435(7044):993.

21. Van de Ven JP, Nilsson SC, Tan PL, Buitendijk GH, Ristau T, Mohlin FC, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. Nat Genet 2013; 45:813-7.

22. Hohenester, E., Sasaki, T. & Timpl, R. Crystal structure of a scavenger receptor cysteine-rich domain sheds light on an ancient superfamily. Nat Struc Mol Biol 1999; 6, 228–32.

23. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.

24. Swaroop A., Chew EY, Rickman CB, Abecasis GR. Unraveling a multifactorial late-onset disease: from genetic susceptibility to disease mechanisms for age-

related macular degeneration. Annu Rev Genomics Hum Genet 2009;10: 19-43.

25. Nozaki M, Raisler BJ, Sakurai E, Sarma JV, Barnum SR, Lambris JD, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. Proc Natl Acad Sci USA 2006;103(7):2328–33.
26. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 2000;14(7):835–46.
27. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma Complement Components and Activation Fragments: Associations with Age-Related Macular Degeneration Genotypes and Phenotypes. Investig ophthalmol Vis Sci 2009;50(12): 5818-27.
28. Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci USA 2010;107(16):7395–400.
29. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet 2009;17: 100–4.
30. Ennis S, Gibson J, Cree AJ, Collins A, Lotery AJ: Support for the involvement of complement factor I in age-related macular degeneration. Eur J Hum Genet 2010; 18: 15–6.
31. Philip Alexander, Jane Gibson, Angela J. Cree, Sarah Ennis, Andrew J. Lotery; Complement factor I and age-related macular degeneration. Mol Vis 2014; 20: 1253–7.