



# Association of central serous chorioretinopathy with single nucleotide polymorphisms in complement factor H gene in Iranian population

Reza Karkhaneh<sup>1</sup> · Mohsen Toufighi<sup>1</sup> · Akbar Amirfiroozy<sup>2</sup> · Aliasghar Ahmad-Raji<sup>3</sup> · Oveis Ahmadzadeh<sup>4</sup> · Alborz Mahdavi<sup>4</sup> · Morteza Naderan<sup>4</sup> 

Received: 4 August 2020 / Revised: 7 April 2021 / Accepted: 23 April 2021 / Published online: 11 May 2021

© The Author(s), under exclusive licence to The Royal College of Ophthalmologists 2021

## Abstract

**Objectives** To investigate the association of two different single nucleotide polymorphisms (SNPs) in the complement factor H (CFH) gene with central serous chorioretinopathy (CSCR) in the Iranian population.

**Methods** This is a case-control study with 95 participants in each group who were stratified according to their various ethnical variations. Primers for rs1329428 and rs3753394 polymorphisms were synthesized. DNA was extracted from peripheral blood leukocytes and underwent PCR and high-resolution melt analysis.

**Results** The frequency of tt, ct, and cc genotypes for rs1329428 polymorphism was 22 (26.5%), 46 (55.4%), and 15 (18.1%) in acute CSCR and 5 (41.7%), 5 (41.7%), and 2 (16.7%) in chronic CSCR respectively with no significant difference between case and control groups. The frequency of tt, ct, and cc genotypes for rs3753394 polymorphism was 31 (37.3%), 14 (16.9%), and 38 (45.8%) in acute CSCR and 4 (33.3%), 3 (25%), and 5 (41.7%) in chronic CSCR respectively. There was a significant difference between patients of Persian descent and controls in rs3753394 polymorphism ( $P = 0.00$ , chi-square test). There was no statistical difference in the frequency of polymorphism between acute and chronic patients ( $P = 0.64$  and  $P = 0.79$  respectively, chi-square test).

**Conclusions** The rs3753394 polymorphism is probably associated with CSCR in Persian ethnicity. Further studies are required to validate the implications of this finding in clinical practice.

## Introduction

Central serous chorioretinopathy (CSCR) is characterized by sensory retinal detachment in the macular area. It causes vision loss or distortion which may remain after resolution of the detachment. It is the fourth most common retinal disease that mainly affects the adult population [1]. Men are

affected 6–10 times more than women [2]. The chorioretinal pathology can be demonstrated in both eyes, though the clinical presentation is unilateral in two-thirds of patients [3]. It may be acute, with spontaneous resolution of sub-retinal fluid, or chronic with retinal pigment epithelium (RPE) atrophy and photoreceptor damage [4].

The etiology of CSCR is unknown. A number of factors including personality type A, increased steroid levels (endogenous or iatrogenic), obstructive sleep apnea, pregnancy, organ transplantation, connective tissue diseases, alcohol consumption, systemic hypertension, and infections (*H. Pylori*) have been proposed to play part in the pathogenesis of CSCR [5, 6].

In recent years, genetic associations of CSCR have been contemplated [7–9]. It was known from before that CSCR may have familial predisposition [10, 11]. In patients with chronic CSCR, associations have been found between complement factor H gene (CFH) mutations and the pathophysiology of the disease [7, 9]. These mutations create single nucleotide polymorphisms (SNPs) which may

✉ Morteza Naderan  
morteza.naderan@yahoo.com

<sup>1</sup> Department of Vitreoretinal Surgery, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

<sup>2</sup> Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

<sup>3</sup> Department of Ocular Emergency and Trauma, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

<sup>4</sup> Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

affect the promoter of specific genes so as to increase or decrease transcription.

The goal of this study was to investigate the association of two SNPs (including rs3753394 and rs1329428) in the CFH gene (1q31.3) with CSCR in an otherwise healthy Iranian population.

## Materials and methods

This is a case-control study performed in Farabi Eye Hospital, Tehran, Iran. The study was conducted from September 2016 to February 2018. The design of the study was approved by the Tehran University of Medical Sciences' Ethics Committee in accordance with the tenets of the Declaration of Helsinki.

Participants included a non-randomized sample of 95 adult patients diagnosed with acute or chronic CSCR, based on the combination of clinical examinations, optical coherence tomography (OCT), fluorescein angiography (FA), and/or indocyanine green angiography (ICGA). Acute CSCR was defined as the resolution of subretinal fluid within 6 months from the onset of symptoms. Chronic CSCR was defined as the persistence of subretinal fluid beyond 6 months after the diagnosis has been first made by an ophthalmologist. Patients demonstrating recurrent CSCR, or those whose documents for the first presentation were missing, were excluded. We also excluded patients with concomitant ocular or systemic diseases such as diabetes mellitus (DM), connective tissue disease, kidney disease, blood dyskrasias, bleeding diathesis, liver disease, and history of systemic infection in the last three months were excluded. Patients with significant cataracts that interfered with the acquisition of high-quality images were excluded as well.

The control group was selected from otherwise healthy subjects presented to the optometry clinic for assessment of visual acuity. This group was consulted by an ophthalmologist for the history of visual symptoms and confirmation of normal retinal examination and OCT images. In the presence of subretinal fluid or pigment epithelial detachments (PED), participants were excluded from the control group. All patients were presented with detailed information about the scientific nature of the study and, after documentation of the informed consent, were registered for evaluations.

The population of the study was stratified into one of the following five ethnic variations according to their own statement of having at least three maternal and paternal ancestors within the same ethnicity: Turkish, Kurdish, Lor, Persian, and mix.

In order to extract DNA, 5 ml of peripheral blood was obtained and preserved at  $-80^{\circ}\text{C}$ . DNA was extracted by a

genomic extraction kit (Pishgaman Inc., Tehran, Iran) according to the manufacturer's protocol. The quality and quantity of the extracted DNA were evaluated by matrix-assisted laser mass spectrometry. Primer design and synthesis were accomplished with the help of Primer 3 software, OligoAnalyzer tool, and UCSC website by Takapouzist company (Takapouzist Inc., Tehran, Iran). The forward and reverse primers for rs1329428 were 5'TCAGTGGTCTAGATAGAGACTCTGGAT3' and 5'TCACTCTTAGAACAAGTTTGTCCAC3' respectively. The forward and reverse primers for rs3753394 were 5'TGAGGT TTATACACAATAGACCCGA3' and 5'AGGGAAATTCTC CGTTGGAAA3' respectively. Polymerase chain reaction (PCR) was performed using an automated machine (Analytik Jena, Jena, Germany). The PCR products underwent agarose gel electrophoresis. Finally, the high-resolution melt (HRM) analysis was performed (Corbett Rotor Gene 6000, QIAGEN, Hilden, Germany) to detect specific polymorphisms.

The statistical analysis was performed with SPSS version 22 for windows. *P* values below 0.05 were considered significant.

## Results

The total number of 190 subjects divided into two equal groups was analyzed. The CSCR group included 60 (63%) males and 35 (37%) females. The control group included 54 (57%) males and 41 (43%) females. There was no statistical difference in the gender between the two groups. The mean age of the CSCR and control groups were  $41.15 \pm 8.08$  (range: 28–58 years) and  $69.73 \pm 8.44$  (range: 38–85 years) respectively ( $P < 0.001$ ; Mann–Whitney *U* test). The mean best-corrected visual acuity (BCVA) was significantly more in the control group ( $0.09 \pm 0.02$  vs.  $0.42 \pm 0.12$  logMAR;  $P = 0.02$ ; Mann–Whitney *U* test). Yet, there was no difference in the BCVA between patients with rs1329428 and rs3753394 polymorphisms ( $0.43 \pm 0.09$  vs.  $0.41 \pm 0.11$  logMAR respectively;  $P = 0.91$ ; Mann–Whitney *U* test). The mean subfoveal choroidal thickness was significantly more in the CSCR group ( $348 \pm 26 \mu\text{m}$  vs.  $271 \pm 17 \mu\text{m}$  respectively;  $P = 0.03$ ; Mann–Whitney *U* test).

The most prevalent ethnicities in the CSCR group were Persian (71 [75%]), followed by Turkish (10 [10.5%]), Kurdish (8 [8.5%]), Lor (3 [3%]), and mix (3 [3%]) descendants. In the control group Persians accounted for 38 (40%), followed by Turkish (32 [33%]), Kurdish (9 [9.5%]), Lor (3 [3%]), and mix (13 [13.5%]) descendants. We found a significant difference in various ethnicities between the two groups ( $P < 0.001$ , Chi-2 test).

The frequency of tt, ct, and cc genotypes for rs1329428 polymorphism was 22 (26.5%), 46 (55.4%), and 15 (18.1%) in acute CSCR and 5 (41.7%), 5 (41.7%), and 2 (16.7%) in chronic CSCR respectively. The frequency of tt, ct, and cc

**Table 1** The frequency of various SNP genotypes in CSCR and control groups.

	rs1329428							rs3753394							
	CSCR <i>N</i> (%)			Control <i>N</i> (%)			<i>P</i> value*	CSCR <i>N</i> (%)			Control <i>N</i> (%)			<i>P</i> value*	
	tt	ct	cc	tt	ct	cc		tt	ct	cc	tt	ct	cc		
Sex															
Males	16	30	14	16	25	13	0.91	23	12	28	9	25	20	0.00	
Females	11	21	3	8	23	10	0.14	12	5	15	4	22	15	0.00	
Ethnicity															
Turkish	2 (20)	8 (80)	–	9 (28)	13 (40)	10 (31)	0.05	2 (20)	3 (30)	5 (50)	5 (16)	12 (37)	15 (47)	0.89	
Lor	–	3 (100)	–	1 (33)	1 (33)	1 (33)	0.22	1 (33)	–	2 (67)	–	2 (67)	1 (33)	0.18	
Kurdish	3 (37)	4 (50)	1 (13)	2 (22)	5 (57)	2 (22)	0.74	3 (37)	2 (25)	3 (35)	3 (33)	3 (33)	3 (33)	0.93	
Persian	22 (31)	34 (48)	15 (21)	10 (26)	22 (58)	6 (16)	0.59	28 (40)	12 (17)	31 (44)	4 (10)	20 (52)	14 (37)	0.00	
Mix	–	2 (67)	1 (33)	2 (15)	7 (53)	4 (31)	0.76	1 (33)	–	2 (67)	2 (12)	10 (62)	2 (15)	0.04	

\*Based on chi-square test.

genotypes for rs3753394 polymorphism was 31 (37.3%), 14 (16.9%), and 38 (45.8%) in acute CSCR and 4 (33.3%), 3 (25%), and 5 (41.7%) in chronic CSCR respectively. There was no statistical difference in the prevalence of either polymorphism between acute and chronic patients ( $P = 0.54$  and  $P = 0.79$  respectively, chi-square test).

Patients of Persian descent showed a significant difference in the frequency of rs3753394 polymorphism in comparison to the control group. None of our subpopulations showed a significant difference in the frequency of rs1329428 with the control group (see Table 1).

## Discussion

We performed this study to investigate the association of SNPs in the CFH gene with CSCR and we found that rs3753394 was significantly more common in patients with CSCR in comparison to the control group and the rs1329428 was not associated with CSCR. To the best of our knowledge, this is the first study with the Iranian population.

CSCR is considered an idiopathic entity with numerous environmental associations [5, 6]. However, in recent years much interest has been paid to investigating genetic associations of CSCR [7–9]. For a number of reasons, the CFH gene is a good candidate for association studies. First, the product of the CFH gene is a protein with 20 domains that are secreted into the bloodstream and binds to a calcitonin-related protein (adrenomedullin) which finally results in dilatation of choroidal vasculature, and presumably, increased permeability [12, 13]. Second, proteomic and metabolomic studies on the subretinal fluid in CSCR patients have shown that CFH may have been upregulated [14]. Breukink and colleagues have shown that those with an absent genomic copy of the C4b factor have a greater risk of chronic CSCR while those with three copies have a lower risk [15]. Third, the rs3753394 is

located upstream of the CFH gene and its polymorphisms may theoretically affect the downstream gene's product. Although it is proposed that SNPs may affect the regulatory functions of CFH gene products, it has not been proved whether this influence is exerted through qualitative or quantitative changes [16]. Fourth, the CFH gene has been shown to be associated with age-related macular degeneration (AMD), another retinal disease that shares many common features with CSCR [9, 17–20]. It has been suggested that the same CFH alleles may confer risk for AMD while protecting against CSCR [7, 9, 21].

In a study with Japanese participants, five CSCR-associated SNPs have been determined, including rs800292, rs3753394, rs2284664, rs1329428, and rs1065489 [9]. In our study, we only searched for rs1329428 and rs3753394. The former was not associated with CSCR, while, the latter was more common in the CSCR group. Another study in a Greek population demonstrated three SNPs associated with CSCR: rs3753394, rs1329428, and rs1065489 [8]. We only found the first polymorphism as a risk factor. Other investigators also reported CSCR-associated SNPs in the European population, including rs800292, rs1329428, and rs1065489 [7]. In the largest-scale study of this kind, six CSCR-associated SNPs have been evaluated in the Chinese population comprising the following polymorphisms: rs800292, rs1061170, rs3753396, rs2284664, rs1329428, and rs1065489 [16].

It is noteworthy that not all SNPs are essentially risk factors for disease development. Indeed, some of them may offer protection. For example, rs1065489 was found to protect against CSCR in Japanese, Chinese, as well as West European populations [7, 9, 16], while it was found as a predisposing association among Greek patients [8]. The rs800292 has been shown to be a risk factor for CSCR in the Chinese population, however, it was not associated with CSCR in Greek patients [8, 16]. Likewise, we found that rs3753394 has associated with CSCR especially in the

Persian descent subpopulation. It appears that these controversies stem from racial differences [22]. Hence, large-scale studies with independent cohorts and replication studies are required to reach definitive conclusions. Furthermore, it is not known whether any of these polymorphisms influence the response to treatment [23].

The sample size is a very important issue if association studies are to be reliable. For example, Linglu and colleagues reported no association of rs1329428 and rs2284664 with CSCR in their Chinese population. Nevertheless, their meta-analysis demonstrated that these two SNPs are associated with CSCR [16]. Furthermore, looking for associations in the CFH gene locus is due to the current recognition of this area as a possible candidate for CSCR development. Nonetheless, there may be important, yet unrecognized, loci that affect the susceptibility. In fact, there are only three published genome-wide association studies (GWAS) that have linked the CFH gene to CSCR [21, 24, 25]. Unfortunately, most of these studies lack enough large sample sizes and independent cohorts that can provide reliable replication studies, thus limiting their conclusions. Recently, Hosoda and colleagues have published the results of their large-scale GWAS in Japanese and European populations. They have identified rs6061548 and rs13278062 polymorphisms which have not been identified as risk factors for CSCR in previous studies. The former is not in the CFH gene territory, while the latter, is located in the CFH locus and previously has been mapped as a risk factor for AMD [26]. This new polymorphism should be studied further to see whether it increases the risk for secondary choroidal neovascularization (CNV) in CSCR patients, or another similar entity that shares features of both AMD and CSCR, the so-called pachychoroidal neovascularopathy (PCNV) [21].

The main limitation of our study is its small sample size. Although we subdivided the population into different Iranian ethnical variations, the total number of each subgroup was relatively small for firm conclusions to be made.

Finally, it seems that the association of various SNPs in the CFH gene locus with CSCR merit further evaluation. Understanding these polymorphisms helps determine the pathologic basis of CSCR accurately and may also have implications in the treatment modalities that develop in the future.

## Summary

### What is known about this topic

- Complement factor H gene polymorphisms have been associated with an increased risk of central serous chorioretinopathy (CSCR).

- There have been contradicting reports of polymorphisms in different races that could predispose or protect against CSCR.
- There have been no reported polymorphisms in Iranian populations with CSCR.

### What this study adds

- The rs3753394 polymorphism is more common in patients of Persian descent in comparison to the control group.
- The frequency of rs1329428 polymorphism is not different between patients and control groups.

**Author contributions** RK, MT, MN, and AA were responsible for designing the study protocol; MT, AAR, OA, and MN were responsible for case selection and recruitment; AA and MT were responsible for genetic analyses; MT, MN, AM, and OA were responsible for data analysis; MN, AM, and MT were responsible for primary draft preparation; RK, AA, and AAR were responsible for feedback on the primary draft and supervision of appropriate modifications; MN was the corresponding author and responsible for accomplishing suggested revisions.

**Funding** This work has been funded by the Tehran University of Medical Sciences Grants for the ophthalmology residency program.

### Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

1. Wang M, Munch IC, Hasler PW, Prunte C, Larsen M. Central serous chorioretinopathy. *Acta Ophthalmol.* 2008;86:126–45.
2. Polak BC, Baarsma GS, Snyers B. Diffuse retinal pigment epitheliopathy complicating systemic corticosteroid treatment. *Br J Ophthalmol.* 1995;79:922–5.
3. Taban M, Boyer DS, Thomas EL, Taban M. Chronic central serous chorioretinopathy: photodynamic therapy. *Am J Ophthalmol.* 2004;137:1073–80.
4. Leveque TK, Yu L, Musch DC, Chervin RD, Zacks DN. Central serous chorioretinopathy and risk for obstructive sleep apnea. *Sleep Breath.* 2007;11:253–7.
5. Kitzmann AS, Pulido JS, Diehl NN, Hodge DO, Burke JP. The incidence of central serous chorioretinopathy in Olmsted County, Minnesota, 1980–2002. *Ophthalmology.* 2008;115:169–73.
6. Liew G, Quin G, Gillies M, Fraser-Bell S. Central serous chorioretinopathy: a review of epidemiology and pathophysiology. *Clin Exp Ophthalmol.* 2013;41:201–14.
7. de Jong EK, Breukink MB, Schellevis RL, Bakker B, Mohr JK, Fauser S. et al. Chronic central serous chorioretinopathy is associated with genetic variants implicated in age-related macular degeneration. *Ophthalmology.* 2015;122:562–70.

8. Moschos MM, Gazouli M, Gatzoufas Z, Brouzas D, Nomikarios N, Sivaprasad S, et al. Prevalence of the complement factor H and GSTM1 genes polymorphisms in patients with central serous chorioretinopathy. *Retina* 2016;36:402–7. <https://doi.org/10.1097/IAE.0000000000000693>.
9. Miki A, Kondo N, Yanagisawa S, Bessho H, Honda S, Negi A. Common variants in the complement factor H gene confer genetic susceptibility to central serous chorioretinopathy. *Ophthalmology*. 2014;121:1067–72.
10. Weenink AC, Borsje RA, Oosterhuis JA. Familial chronic central serous chorioretinopathy. *Ophthalmologica*. 2001;215:183–7.
11. Oosterhuis JA. Familial central serous retinopathy. *Graefes Arch Clin Exp Ophthalmol*. 1996;234:337–41.
12. Udono-Fujimori R, Udono T, Totsune K, Tamai M, Shibahara S, Takahashi K. Adrenomedullin in the eye. *Regul Pept*. 2003;112:95–101.
13. Dorner GT, Garhöfer G, Huemer KH, Golestani E, Zawinka C, Schmetterer L, et al. Effects of adrenomedullin on ocular hemodynamic parameters in the choroid and the ophthalmic artery. *Investig Ophthalmol Vis Sci*. 2003;44:3947–51.
14. Kowalczyk L, Matet A, Dor M, Bararpour N, Daruich A, Dirani A, et al. Proteome and metabolome of subretinal fluid in central serous chorioretinopathy and rhegmatogenous retinal detachment: a pilot case study. *Transl Vis Sci Technol*. 2018;7:3 <https://doi.org/10.1167/tvst.7.1.3>.
15. Breukink MB, Schellevis RL, Boon CJF, Fauser S, Hoyng CB, den Hollander AI, et al. Genomic copy number variations of the complement component C4B gene are associated with chronic central serous chorioretinopathy. *Investig Ophthalmol Vis Sci*. 2015;56:5608–13.
16. Linglu D, Xu h, Cao Y, Qu J, Jin E, Gao T, et al. Association of central serous chorioretinopathy with single-nucleotide polymorphisms in complement factor H Gene in a Chinese population. *BMC Ophthalmol*. 2020. <https://doi.org/10.21203/rs.2.11402/v1>. (preprint version).
17. Dong L, Qu Y, Jiang H, Dai H, Zhou F, Xu X, et al. Correlation of complement factor H gene polymorphisms with exudative age-related macular degeneration in a Chinese cohort. *Neurosci Lett*. 2011;488:283–7.
18. 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 USA*. 2005;102:7227–32. <https://doi.org/10.1073/pnas.0501536102>.
19. Hughes AE, Bridgett S, Meng W, Li M, Curcio CA, Stambolian D, et al. Sequence and expression of complement factor H gene cluster variants and their roles in age-related macular degeneration risk. *Investig Ophthalmol Vis Sci*. 2016;57:2763–9. <https://doi.org/10.1167/iovs.15-18744>
20. van Asten F, Simmons M, Singhal A, Keenan TD, Ratnapriya R, Agrón E, et al. A Deep phenotype association study reveals specific phenotype associations with genetic variants in age-related macular degeneration: age-related eye disease study 2 (AREDS2) report no. 14. *Ophthalmology*. 2018;125:559–68. <https://doi.org/10.1016/j.ophtha.2017.09.023>.
21. Hosoda Y, Yoshikawa M, Miyake M, Tabara Y, Ahn J, Woo SJ, et al. CFH and VIPR2 as susceptibility loci in choroidal thickness and pachychoroid disease central serous chorioretinopathy. *Proc Natl Acad Sci USA*. 2018;115:6261–6. <https://doi.org/10.1073/pnas.1802212115>.
22. Chan WM, Lim TH, Pece A, Silva R, Yoshimura N, Verteporfin PDT. for non-standard indications-a review of current literature. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:613–26. <https://doi.org/10.1007/s00417-010-1307-z>.
23. Linghu D, Xu H, Liang Z, Gao T, Lin Z, Li X, et al. Association between CFH single nucleotide polymorphisms and response to photodynamic therapy in patients with central serous chorioretinopathy. *Int Ophthalmol*. 2020;40:951–6. <https://doi.org/10.1007/s10792-019-01261-y>.
24. Schellevis RL, van Dijk EHC, Breukink MB, Altay L, Bakker B, Koeleman BPC, et al. Role of the complement system in chronic central serous chorioretinopathy: a genome-wide association study. *JAMA Ophthalmol*. 2018;136:1128–36. <https://doi.org/10.1001/jamaophthalmol.2018.3190>.
25. Miki A, Sakurada Y, Tanaka K, Semba K, Mitamura Y, Yuzawa M, et al. Genome-wide association study to identify a new susceptibility locus for central serous chorioretinopathy in the Japanese population. *Investig Ophthalmol Vis Sci*. 2018;59:5542–7. <https://doi.org/10.1167/iovs.18-25497>.
26. Arakawa S, Takahashi A, Ashikawa K, Hosono N, Aoi T, Yasuda M, et al. Genome-wide association study identifies two susceptibility loci for exudative age-related macular degeneration in the Japanese population. *Nat Genet*. 2011;43:1001–4. <https://doi.org/10.1038/ng.938>.