BJMG 13/2 (2010) 5-10
10.2478/v10034-010-0025-5

ORIGINAL ARTICLE

COMPLEMENT FACTOR H Y403H POLYMORPHISM IN THE TURKISH POPULATION

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ABSTRACT

Complement factor H (*CFH*) is an important regulator protein of the alternative pathway of the complement system. The *CFH* mutations and polymorphisms in CFH have been associated with diseases of the kidney and eye. We investigated the allelic frequency of the most common *CFH* polymorphism, c.1277 T>C (Y402H), in 100 healthy Turkish volunteers from the Antalya Province by direct sequencing of the corresponding genomic region. We found a frequency of 0.65% for the T and 0.35% for the C alleles. The frequency of the TT, CT and CC genotypes was 0.40, 0.49 and 0.11% respectively. Thus, the disease-related C allele has a frequency in Turkey similar to that of Caucasian populations.

Key words: Complement factor H (*CFH*), c.1277 T>C, Polymorphism, Y402H.

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INTRODUCTION

Complement factor H (*CFH*) is an important member of the regulator of complement activation protein family of innate immunity. The *CFH*, a plasma glycoprotein or attached to outer cell membrane, restricts the activation of complement on the host cell membrane and protects tissues from damage produced by complement activation [1,2].

The *CFH* gene is located on chromosome 1q32 and encodes 23 exons for a 155kDa glycoprotein [2-4]. More than 550 single nucleotide polymorphisms (SNPs) of the gene have been discovered (www.ncbi.nih.gov/SNP). The c.1277 T>C (Y402H) is a well known polymorphism that leads to substitution of tyrosine by histidine at codon 402. This polymorphism is located on the short consensus repeat 7 (SCR-7) domain of the *CFH* protein that binds the C-reactive protein (CRP), sialic acid and heparin [2,5-7].

The *CFH* mutations have been associated with adult or atypic hemolytic uremic syndrome (aHUS), HUS, membrano proliferative glomerulo nephritis 2 (MPGN2), age-related macular degeneration (AMD) and basal laminar drusen [8-16] Although the c.1277 T>C variant is less common in renal diseases, it is strongly associated with AMD [17,18]. We have determined the frequency of the c.1277 T>C polymorphism of the *CFH* gene in a Turkish population from southwest Turkey.

MATERIALS AND METHODS

We examined 54 females and 46 males (aged 17 to51 years) from the university staff, who declared that they had no health problems. They were all from Antalya Province in Turkey and all gave their informed consent.

Genomic DNA was isolated from whole peripheral blood samples by the classic salting-out procedure [19]. The region which includes the c.1277 T>C variant in the *CFH* gene was amplified using polymerase chain reaction (PCR) by forward primer 5'-CTT TGT TAG TAA CTT TAG TTC GTC TTC AG-3' and reverse primer 5'-CAA GGT GAC ATA ACA TTT TGC C-3'. Approximately 100 ng of genomic DNA, measured by NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA), was used in 25 μL reaction volumes. The PCR mixture contained: 1.25 U Taq polymerase (GoTaq; Promega, Madison, WI, USA), 10X buffer (pH 8.8 with ammonium sulfate; Fermentas, Vilnius, Lithuania), 1.5 mM $MgCl_2$ (Fermentas), 5 pmol of each primer and 200 mM of each of the four dNTPs. The PCR program consisted of 40 cycles of initial denaturation at 93°C for 150 seconds, 36 cycles of 30 seconds at 93°C, 45 seconds at 58°C, 60 seconds at 72°C, and final extension at 72°C for 5 min. The 443 bp PCR products were electrophoresed on 2.5% agarose gel containing ethidium bromide, and then visualized under UV light using imaging system (SynGene InGenius, Cambridge, Cambridgeshire, UK).

The PCR products were purified by PureLink™ PCR Purification Kit (Invitrogene, Carlsbad, CA, USA). Sequencing was done using the BigDye Terminator v3.1 Cycle Sequencing kit and an ABI PRISM™ 3130 × l Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The allele and genotype frequencies were calculated using the allele counting method.

RESULTS

Direct sequencing of the 443 bp PCR products showed no genomic alteration other than c.1277 T>C. The frequency of the T and C alleles was 0.65 and 0.35%, respectively (Table 1). The TT, CT and CC genotypes had a frequency of 0.40, 0.49 and 0.11%, respectively. Representative samples of the sequences of each genotype are shown in Figure 1.

DISCUSSION

The *CFH*-mediated inactivation of the alternative pathway protects the host cell membranes from excessive complement system activation. The Y402H substitution interferes with the binding ability of the SCR7 domain for

Table 1. Frequency of the *CFH* gene c.1277 T>C alleles in different populations.

Population	Healthy Controls (n)	C Allele Frequency (%)	References
American (Icelandic)	1,265	0.39	26
American	108	0.31	27
American	190	0.36	9
American[a]	275	0.34	22
American[a]	48	0.38	12
European American	403	0.34	10
German	612	0.38	15
French	91	0.30	16
Greek	115	0.37	28
Japanese	107	0.11	29
Turks	100	0.35	This study

[a] Control groups were specifically defined as non Hispanic Whites in these two studies.

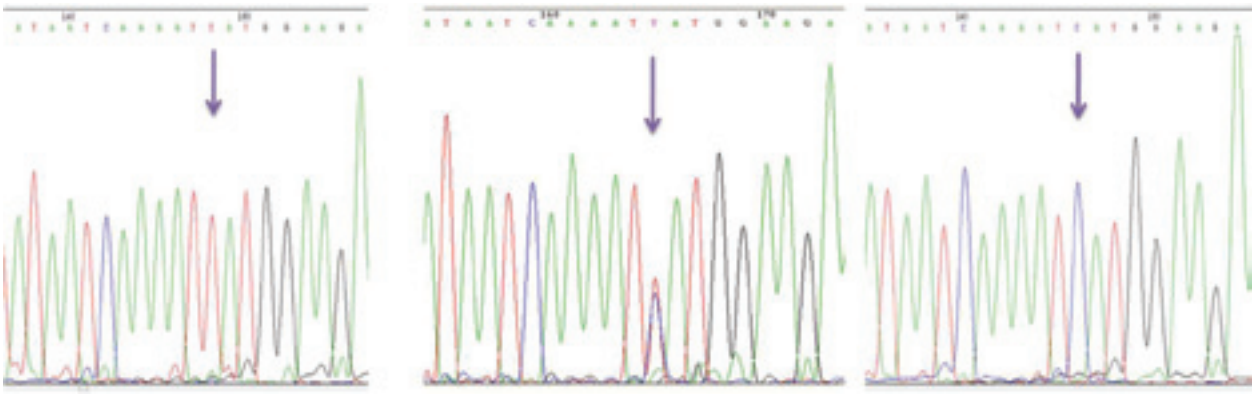

Figure 1. The c.1277 T>C substitution of the *CFH* gene. The arrows indicate the substitution positions and show the TT, TC and CC genotypes.

poly-anionic molecules such as heparin and CRP, which are biological markers of inflammation [6,20]. As a consequence, this variant leads to cell loss and increased damage of target tissues. The *CFH* mutations and variants have been associated with renal and ocular conditions like MPGN2, aHUS, basal laminar drusen, and AMD. Sometimes patients may have more than one of these conditions, suggesting common mechanisms in their pathogenesis [1]. However, there is no straightforward genotype-phenotype correlation between the *CFH* variants and disease [1]. It has been reported that the C allele for the *CFH* c.1277 T>C polymorphism increases the odds ratio of AMD up to 6-fold in different populations except the Japanese [21-23]. An 8-fold increased risk of developing AMD has been reported for the CC genotype with a positive family history of AMD [16]. Ethnic differences in disease-susceptible genomic alterations have also been shown for the *CFH*-related phenotypes [24,25]. While the C allele frequency varies between 0.30 and 0.39% in different Caucasian populations, its frequency is 0.11% for Japanese people [24,29]. We found the C allele frequency to be 0.35% in our Turkish population (Table 1). This C allele frequency makes it important, especially for AMD in Turkey. Our experimental results revealed no other SNPs in the region comprising the SCR-7 domain of the *CFH* gene. This may imply that the SCR-7 domain is a conserved and func-tionally-important region of the *CFH* gene.

ACKNOWLEDGMENTS

This study was supported by Akdeniz University Scientific Projects Management Units, Antalya, Turkey.

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