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Complement factor H polymorphisms in Japanese population with age-related macular degeneration

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Purpose: To study the frequency of five haplotypes previously reported in the complement factor H (*CFH*) gene for Japanese patients with age-related macular degeneration (AMD).
Methods: Genomic DNA was isolated from peripheral blood samples taken from 96 Japanese AMD patients and 89 age-matched controls. All patients were diagnosed as having exudative (wet-type) AMD. The amplified polymerase chain reaction (PCR) products of *CFH* exons 2, 9, and 13, and intron 6 were analyzed by temperature gradient capillary electrophoresis (TGCE) and by direct sequencing. The haplotypes were identified, and their frequencies were calculated and compared with reported results.
Results: Five haplotypes were identified in the Japanese population including four already reported in the American population. The frequencies of these haplotypes were significantly different between Japanese and American in both control and case groups. The haplotype containing Y402H, which was previously reported to be associated with AMD, was only 4% in the control and case population, with a p value of 0.802. However, two other haplotypes were found as risk factors, which gave an increased likelihood of AMD of 1.9 and 2.5 fold (95% CI 1.12-3.69 and 1.42-6.38). One protective haplotype that decreased the likelihood of AMD by 1.6 fold (95% CI 0.26-0.67) was identified.
Conclusions: The frequencies for five haplotypes previously identified were analyzed in a Japanese population with AMD. Four previously found haplotypes were identified and one additional haplotype was found. The frequencies of each haplotype were significantly different from that in found Americans affected with AMD. Two of the haplotypes were identified as risk factors and one was considered protective.

Age-related macular degeneration (AMD) is the leading cause of visual disability in the elderly in developed countries or in those with a predominant Caucasian population. The disease is characterized by poor vision in the central field due to a progressive destruction of the macular area. A hallmark of the early stage of AMD is the development of drusen, a complex mixture of proteins and cellular deposits, which accumulate between the retinal pigment epithelium (RPE) and Bruch's membrane. Recent studies have shown that drusen are composed of proteins related to activated complement and suppressor [1,2].

Recently, five studies reported that a tyrosine to histidine change at amino acid 402 (Y402H has a T to C substitution at nucleotide 1277 in exon 9) of the complement factor H (*CFH*) gene was strongly associated with AMD [3-7]. Although the frequency of the C allele was not the same in all the reports, it was calculated to be between 0.61-0.94 in AMD cases and 0.34-0.46 in age-matched controls [5,7].

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The *CFH* gene, located on chromosome 1q32, was the first of 13 distinct loci mapped on 11 chromosomes to be identified as being associated with AMD [8,9]. CFH functions as a cofactor in the inactivation of C3b by factor I, and it also increases the rate of dissociation of the C3bBb complex and the NBB complex in the alternative complement pathway [10]. CFH is also known to be decreased in individuals who smoke, the greatest risk factor for AMD [11].

The purpose of this study was to analyze the five haplotypes previously identified in the *CFH* gene in a Japanese population with exudative AMD and in age-matched controls.

METHODS

Age-related macular degeneration patients and age-matched controls: Blood samples were collected from 96 Japanese patients with exudative (wet type) AMD and 89 age-matched controls between 50-85 years old. AMD was diagnosed by ophthalmoscopic and fluorescein angiographic findings. In controls, no signs of early AMD, such as soft drusen or irregular pigmentations of the RPE in the macular area, were observed ophthalmoscopically. Informed consent was obtained from all participants, and the procedures used conformed to the tenets of the Declaration of Helsinki.

DNA extraction, sequence analysis of complement factor H gene, and statistical analysis: DNA was extracted from blood using a DNA isolation kit (QIAamp DNA Blood Maxi Kit, QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) amplification of genomic DNA from AMD patients and controls was performed using gene-specific primers and LA-Taq polymerase (Takara Bio Inc., Ohtsu, Japan). The primers (forward primer: 5'-CCT AGA AAC CCT AAT GGA ATG TG-3', reverse primer: 5'-CCG CTT CAA TAC GAC TTC ATT-3') were designed to amplify exons 8 and 9 of the *CFH* gene in approximately 1.3 Kb size. The amplified products were directly sequenced by CEQ2000XL DNA analysis system using a primer, 5'-CCG CTT CAA TAC GAC TTC ATT-3', and dye terminator sequencing kit (Beckman Coulter, Fullerton, CA). Primers to identify each single nucleotide polymorphism (SNP) I62V (forward primer: 5'-ATA GAC CTG TGA CTG TCT AGG CA-3', reverse primer: 5'-CAG AGC CAG ACT CCA TCT CA-3'), IVS6 (forward primer: 5'-GCA TCT CAT AGC TTT TGA CTT CA-3', reverse primer: 5'-ACT GTG TCC ATT CAG CTC CTA A-3'), and Q672Q (forward primer: 5'-CAA TAT GAA CAC CAT TCT TGA TTG-3', reverse primer: 5'-CAC AGG TAC TCT CCT CCA CTA TG-3'), were designed for amplification of these region. PCR products were analyzed with temperature gradient capillary electrophoresis (TGCE; REVEAL, SpectruMedix, State College, PA) for sequence homozygosity or heterozygosity. Against known sequence templates. In the event of homozygosity, template DNA for one of the alleles was mixed at ratio of 1:1 to repeat the analysis. Statistical analysis was performed using single nucleotide polymorphism (SNP) and disease association software (SNPAlyze version 5.0; DYNACOM, Chiba, Japan).

RESULTS & DISCUSSION

Using the four SNPs reported by Hageman et al. [6], we identified five haplotype blocks including one specific to the Japanese population. The frequency for each haplotype differed significantly from that previously described (Figure 1). Haplotype 5, which contains a T to C nucleotide substitution replacing tyrosine by histidine in exon 9, was found at a frequency of 4% for both controls and AMD cases, indicating no association with Japanese AMD. The genetic frequencies of the T/C allele for cases and controls were 85.42% and 88.76% for T/T homozygosity, 12.50% and 11.24% for T/C heterozygosity, and 2.08% and 0.00% for C/C homozygosity, respectively. The overall frequency of the C allele in cases (8.3%) and controls (5.6%) was significantly lower than that previously reported 61-94% in cases and in 34-46% in controls [3-7].

However, haplotypes 3 and 4 gave a 1.9 fold and 2.5 fold increased likelihood of AMD (1.12-3.69 and 1.42-6.38, respectively, 95% confidence interval [CI]; Figure 1). The frequencies of both haplotypes in the Japanese AMD population were between 15-19% independently. On the other hand, haplotype 2 was found as a protective allele, decreasing likelihood of AMD by 1.6 fold (95% CI 0.26-0.67). Haplotype 1, which was reported as 21% in the American population, gave highest frequency of 35% for both controls and patient cases in the Japanese population.

These results indicated that *CFH* is statistically associated with AMD in the Japanese population. Although the single Y402H allele was not associated with AMD, statistical analysis showed two haplotype blocks were associated with AMD. The frequency of AMD in Japan has been calculated based on

A:

B:

Haplotypes	p	Estimated frequencies: Cases JP	Cases US	Controls JP	Controls US	Risk (R) or protective (P) likelihood (fold JP/US)
H1: G T T G	1.000	0.38	N/A	0.38	N/A	1.0/-
H2: A T T A	0.001	0.18	0.12	0.35	0.21	(P) 1.6/1.7
H3: G T T A	0.028	0.19	N/A	0.10	N/A	(R) 1.9/-
H4: A T T C	0.004	0.15	N/A	0.06	N/A	(R) 2.5/-
H5: G T C A	0.802	0.04	0.50	0.04	0.29	(R) 1.0/1.7

Figure 1. Association analysis of Complement Factor H haplotypes. **A**: Four sets of single nucleotide polymorphisms (SNPs; I62V, IVS6, Y402H, Q672Q) were selected to distinguish all five haplotypes reported [6]. **B**: All haplotypes with a frequency >3% are displayed. The estimated frequencies for Japanese (JP) and American (US) cases and controls in these risk and protective haplotypes are shown (JP/US). The red "C" represents the nucleotide responsible for the Y402H polymorphism. N/A means "not available."

the study in Hisayama district of Kyushu, Japan. The incidence of late stage AMD, defined as the presence of neovascularization and geographic atrophy of the macula, was calculated to be 0.8% of the 1,482 residents [12]. This number is significantly lower than that of the Beaver Dam Eye Study (9% over 65 years of age and 28% for all stages of AMD) and other population studies [13-15]. The sum of frequencies for haplotypes 3 and 4 in AMD cases was 33.32%, indicating that a high proportion of AMD patients have either haplotype, whereas they were 15.6% for control. Our results showed that the responsible alleles and haplotypes for AMD may vary among different ethnic groups. Further analysis to discover new SNPs for *CHF* in the Japanese population may be required to establish further links between this gene and AMD.

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