한국인에서 Complement Factor H 유전자의 변이와
연령관련황반변성의 관련성

Association of Complement Factor H Gene
Polymorphisms with Neovascular Age-related
Macular Degeneration in a Korean cohort

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2008 년 2 월
지도교수 오 중 협

이 논문을 석사학위논문으로 제출함

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이 논문을 김나래의 석사학위논문으로 인정함

2008년 2월  일

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Abstract

**Purpose:** This study was undertaken to investigate the association between the complement factor H (CFH) gene and exudative age-related macular degeneration (AMD) in Korean patients.

**Methods:** Genomic DNA was isolated from the peripheral leukocytes of exudative AMD patients (n=114) and controls (n=187). Criterion for exudative AMD was confined to the presence of choroidal neovascularization. Four single nucleotide polymorphisms (SNPs: -275C>T, I62V, Y402H, IVS15) located in promoter, exon2, exon9, and intron15 of the CFH gene were genotyped by PCR-based direct sequencing.

**Results:** The frequency of the C allele of Y402H (AMD: 10.5%, control: 6.5%) was found to be lower in Koreans than in Caucasians. In the present study, difference between the frequencies of Y402H in cases and controls did not reach statistical significance (P=0.071). However, the frequencies of the major alleles of three SNPs (-275C>T, I62V and IVS15) were significantly different in patients and controls, and these SNPs were found to be separately associated with an elevated risk of exudative AMD. Seven haplotypes were identified in Koreans. Haplotype analysis showed that two haplotypes (TGTG, CGTG) conferred significantly higher risks of exudative AMD (P=0.013, 0.035), and one haplotype (CATA) was significantly protective (P<0.001).

**Conclusion:** In Korean subjects, CFH polymorphism appears to be a considerable hereditary contributor to exudative AMD. Y402H polymorphism which has been suggested to be a major risk factor of AMD in Caucasians was only found to be marginally associated with exudative AMD with low frequency, while three adjacent SNPs in CFH gene were significantly associated with AMD in Koreans.
Key Words: age-related macular degeneration; complement factor H; CFH; mutation; single nucleotide polymorphism
목적: Complement factor H(CHF) 유전자가 한국인의 삼출성 연령관련황반변성과 어떠한 관련성이 있는지 알아본다.

방법: 형광안저혈관조영술을 통해 신생혈관을 동반한 연령관련황반변성을 진단받은 환자군 114 명과 안과적으로 특별한 이상이 없는 대조군 187 명의 정맥혈에서 DNA를 추출하였다. CFH 유전자의 promoter, exon2, exon9, intron15에 위치한 4개의 단일염기다형성 275C>T, I62V, Y402H, IVS15에 대해, 중합효소연쇄반응(PCR)을 기초로 한 직접서열결정(direct sequencing) 방법으로 염기서열을 분석하였다.

결과: 한국인에서 Y402H의 C allele 변도(AMD 환자군: 10.5%, 대조군: 6.5%)는 백인에 비해 낮았고, 환자군과 대조군 간의 Y402H 변도는 통계학적으로 유의한 차이에 도달하지 않았다(P=0.071). 그러나 세 개의 SNPs(275C>T, I62V, IVS15)는 환자군과 대조군 사이에 유의한 변도 차이를 보였으며, 각각 삼출성 연령관련황반변성의 위험을 증가시키는 것으로 나타났다. 7개의 반수체형(haplotype)이 확인되었으며, 반수체형 분석에서 두 개의 반수체형(TGTG, CGTG)이 삼출성 연령관련황반변성을 유의하게 증가시키는 것으로 나타났고(P=0.013, 0.035), 한 개의 반수체형(CATA)이 유의하게 위험도를 감소시키는 것으로 나타났다(P<0.001).

결론: 한국인에서 CFH 유전자 변이는 삼출성 연령관련황반변성에 상당한 관련성이 있는 것으로 보인다. 백인의 삼출성 연령관련황반변성에서 가장 강력한 유전적 요인으로 지목된 Y402H 변이는 한국인을 대상으로 한 이번 연구에서 그 변도가 백인에 비해 매우 낮고, 환자군과 대조군 사이의 차이가 통계학적인 유의성에 도달하지 못한 반면, 주변의 3개의 단일염기다형성은 연령관련황반변성과 유의한 연관성을 보였다.
핵심단어: 연령관련황반변성; complement factor H; CFH; 변이; 단일염기다형성
Introduction

Age-related macular degeneration (AMD) is the primary cause of severe visual loss in the elderly. It commonly occurs in patients older than 50 years and usually affects both eyes.\(^1\) AMD is a genetically complex disorder of the photoreceptor - retinal pigment epithelium (RPE) - Bruch’s membrane - choriocapillaris complex. Early signs of AMD are characterized by the presence of soft drusen, areas of increased pigment or hyperpigmentation (in the outer retina or choroid), and/or areas of depigmentation or hypopigmentation of the RPE. Advanced disease manifests either as geographic atrophy or choroidal neovascularization.\(^2\)

Several risk factors, including aging, cigarette smoking, and arterial hypertension, have been proposed but only a fraction of exudative AMD cases can be attributed to these risk factors.\(^3\) The prevailing view is that AMD is a complex disorder derived from interactions between multiple genetic and environmental risk factors. Genetic influences on AMD have been well established by family and twin studies.\(^4\) However, AMD appears to be the product of interactions between multiple susceptible loci rather than being due to a collection of single-gene disorders.\(^5\)

The Y402H polymorphism in complement factor H (CFH) gene on chromosome 1q31 has been reported to be a risk factor of AMD in North America and Europe.\(^5\textsuperscript{-14}\) However, the majority of these previous studies were performed on predominantly Caucasians of European descent, and it is known that genetic risks of AMD are dependent on ethnicity.\(^15\) Many polymorphisms are potentially associated with AMD development; Y402H is most likely to be associated with AMD in Caucasians, whereas other SNPs have been reported to be associated with AMD in other races, particularly in
Asians. Furthermore, previous studies have suggested that haplotype analysis rather than genotype analysis of the single ‘Y402H’ SNP is required to clarify the association between genetic factors and AMD susceptibility.

In Asia, the prevalence of AMD is increasing rapidly due to population aging. However, no study has demonstrated the association between genetic factors and exudative AMD in Korean patients. Here, we recruited study population of 114 AMD and 187 normal eyes of Koreans in order to examine whether $CFH$ is a major genetic determinant of AMD in Korean. We evaluated the relationships between four common polymorphisms in $CFH$ gene, namely, -275C>T, I62V, Y402H and IVS15 (SNP ID: rs3753394, rs800292, rs1061170, and rs1329428) and AMD in Koreans.
Methods

1. Subjects

This prospective case-control study was approved by the institutional review board (IRB) of Inha University Hospital, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers. Based on history-taking findings, all study subjects resided in the same geographic area and had similar lifestyles, in terms of likely exposure to sunlight, alcohol use, and physical exercise.

Three general hospitals participated, i.e., Inha University Hospital, Severence Hospital, and Wonju Christian Hospital in Korea. All individuals were enrolled at these three centers using the same protocol. Inclusion criteria were as follows: (1) Age > 50 years; (2) a diagnosis of exudative AMD after undergoing comprehensive ophthalmic examinations, which included fundus fluorescein angiography; and (3) the presence of a choroidal neovascular membrane in either or both eyes for a diagnosis of ‘exudative’ disease. Exclusion criteria included the presence of geographic atrophy (GA) or a large drusen in the absence of choroidal neovascularization. Secondary choroidal neovascular diseases, such as, degenerative myopia, angiod streaks, idiopathic choroidal neovascularization, and presumed ocular histoplasmosis syndrome, were excluded based on clinical presentations and angiographic manifestations.

Unrelated control subjects were recruited from among patients with no macular pathology or any other eye disease, except for mild senile cataract, and were more than

3
50 years of age. In addition, controls had no known family history of AMD. Fundus photography was performed routinely on all control subjects.
2. Genotyping

Peripheral venous blood was collected from all subjects into 5-mL EDTA tubes. Genomic DNA was extracted from leukocytes using commercially available QIAmpDNA Blood kits (Qiagen, Valencia, CA), and polymorphic sites were amplified by PCR using specific primers, namely: for -275C>T in the CFH promoter, 5’-ATT
TGT TGA TTT TTG GAT TAT TAA-3’ (forward primer) and 5’-GCT CTT GGA CTT
TTT CTT ATT-3’ (reverse primer); I62V in exon2, 5’-TGC ACT TAT TTT GTT TTT
ATT GTT TGT A-3’ (forward) and 5’-CCA TCT CTA CTA AAA ATA CAA AAA T-3’ (reverse); Y402H in exon9, 5’-ACA GGA GAA ATA AAT ATA GGG ACT T-3’
(forward) and 5’-AAC ATG CTA GGA TTT CAG AGT A-3’ (reverse); and IVS15 in
intron15, 5’-ATC CAG GTA CAT TAA TCA CTC TT-3’ (forward) and 5’-CTG GAG
TTT TTA CAT AGC ATT TTA-3’ (reverse).

Genotype determination was conducted using Bigdye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA) on an ABI 3730 automated sequencer (Applied Biosystems) by PCR-direct sequencing using the primers; 5’-TGA
TTT TTG GAT TAT TAA-3’, 5’-TAT TTT GTT TTT ATT GTT-3’, 5’-GAA ATA
AAT ATA GGG ACT-3’, and 5’-AGG TAC ATT AAT CAC TCT-3’ for -275C>T, I62V, Y402H, and IVS15, respectively.
3. Statistics

Allele and genotype frequencies were estimated using the allele counting method. Hardy-Weinberg estimates for genotypes and estimated haplotype frequencies were calculated using HapAnalyzer ver. 1.0 (http://hap.ngri.go.kr/right.html).

Numerical data were analyzed using the student’s t-test. Associations between several known risk factors and AMD were examined using separate logistic regression models. These known risk factors were treated as categorical variables, namely, age (50-59: 0, 60-69: 1, ≥70 years: 2), gender (male: 0, female: 1), smoking status (nonsmoking: 0, <20 pack-years: 1, ≥20 pack-years: 2), and hypercholesterolemia (<200: 0, ≥200: 1).

The allele and genotype frequencies of cases and controls were compared using chi-square analysis. Chi-square analysis was used to calculate the unadjusted odds ratios of alleles and genotypes. Conditional logistic regression analysis models were considered for each locus separately to estimate odds ratios and their corresponding 95% confidence intervals (CIs), after adjusting for other risk factors. All genotypes were set as categorical variables (homozygotes for the risk allele: 2, heterozygotes: 1, no risk allele: 0).

Pairwise linkage disequilibrium (LD, D’) estimations between SNPs at the CFH locus, and EM-based haplotype association analysis were performed using HapAnalyzer software. All of haplotypes with a frequency of >1% were selected. ORs for the cases and controls were calculated, and 95% CIs were calculated. Homozygotes and heterozygotes for these haplotypes among cases and controls were compared using the chi-square test.
Statistical analysis was performed using SPSS statistical software for Windows (ver. 12.0; SPSS Inc., Chicago, IL). The criterion for statistical significance was P≤0.05.
Results

1. Participants

In total, 114 exudative AMD patients and 187 control subjects participated in this study. The baseline characteristics of patients and controls are shown in Table 1. The mean ages (P=0.148) and gender distributions (P=0.127) of the patients and controls were not significantly different.

Of the putative risk factors, aging (P=0.027) and cigarette smoking (P=0.019) were found to be significantly associated with AMD, whereas no significant association was found for gender (P=0.128), hypercholesterolemia (P=0.111), or hypertension (P=0.126). Hypercholesterolemia and hypertension tended to be more prevalent in patients than in controls (Table 2).
Table 1. Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=114)</th>
<th>Controls (n=187)</th>
<th>All subjects (n=301)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages (yrs)*</td>
<td>68.25 ± 8.806</td>
<td>66.83 ± 7.794</td>
<td>67.37 ± 8.207</td>
<td>P=0.148</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (41.2)</td>
<td>94 (50.3)</td>
<td>141 (46.8)</td>
<td>P=0.127</td>
</tr>
<tr>
<td>Female</td>
<td>67 (58.8)</td>
<td>93 (49.7)</td>
<td>160 (53.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Cases and controls were matched for age and sex.

† Mean ± standard deviation.

Unless otherwise indicated data are expressed as numbers (percentages) of subjects.
Table 2. Characteristics of variables

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases (n=114)</th>
<th>Controls (n=187)</th>
<th>$P^*$</th>
<th>P</th>
<th>ORs (95% CI)$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>15 (13.2)</td>
<td>33 (17.6)</td>
<td></td>
<td></td>
<td>1.000 (Reference)</td>
</tr>
<tr>
<td>60-69</td>
<td>48 (42.1)</td>
<td>99 (52.9)</td>
<td></td>
<td>0.857</td>
<td>1.067 (0.529-2.150)</td>
</tr>
<tr>
<td>≥70</td>
<td>51 (44.7)</td>
<td>55 (29.4)</td>
<td>0.027</td>
<td>0.052</td>
<td>2.040 (0.993-4.189)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (41.2)</td>
<td>94 (50.3)</td>
<td></td>
<td></td>
<td>1.000 (Reference)</td>
</tr>
<tr>
<td>Female</td>
<td>67 (58.8)</td>
<td>93 (49.7)</td>
<td>0.128</td>
<td>0.128</td>
<td>1.441 (0.900-2.306)</td>
</tr>
<tr>
<td><strong>Hypertension$^\ddagger$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>39 (34.2)</td>
<td>79 (43.2)</td>
<td></td>
<td></td>
<td>1.000 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>75 (65.8)</td>
<td>104 (56.8)</td>
<td>0.126</td>
<td>0.126</td>
<td>1.461 (0.899-2.373)</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>33 (28.9)</td>
<td>71 (38.0)</td>
<td></td>
<td></td>
<td>1.000 (Reference)</td>
</tr>
<tr>
<td>≥200</td>
<td>81 (71.1)</td>
<td>116 (62.0)</td>
<td>0.111</td>
<td>0.111</td>
<td>1.502 (0.910-2.480)</td>
</tr>
<tr>
<td><strong>Cigarette smoking (pack-years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>60 (52.6)</td>
<td>128 (68.4)</td>
<td></td>
<td></td>
<td>1.000 (Reference)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>24 (21.1)</td>
<td>30 (16.0)</td>
<td>0.090</td>
<td></td>
<td>1.707 (0.920-3.167)</td>
</tr>
<tr>
<td>≥20</td>
<td>30 (26.3)</td>
<td>29 (15.5)</td>
<td>0.019</td>
<td>0.009</td>
<td>2.207 (1.217-4.003)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval; ORs, odds ratios.

$^*$ Indicates P value (chi-square test) for frequency.

$^\ddagger$ ORs and 95% CIs were determined by logistic regression analysis.

$^\ddagger$ Sample sizes sum to slightly less than the total number of cases and controls because a small amount of covariate data was unavailable.
2. Association between the 4 SNPs and AMD

The genotype frequency distributions of patients and controls were in Hardy-Weinberg equilibrium. As shown in Table 3, the frequency of the Y402H C allele, which has been suggested to be a major risk allele of AMD in North America, was relatively low in patients and controls. The different Y402H frequencies of cases and controls did not reach statistical significance (P=0.071) (10.5% in exudative AMD cases, and 6.5% in controls). Moreover, the Y402H C allele was not significantly associated with AMD but tended to be associated with susceptibility to AMD (OR: 1.716, 95% CI: 0.950-3.100). The allele frequencies of the other three SNPs flanking Y402H were significantly different in patients and controls. The major alleles of the three SNPs (T for -275C>T, G for I62V, and G for IVS15) conferred 1.576 (95% CI: 1.131-2.195, P=0.007), 2.205 (95% CI: 1.553-3.132, P<0.001), 1.997 (95% CI: 1.426-2.797, P<0.001) fold increases in the likelihood of exudative AMD, respectively. After adjusting for known risk factors, significant associations were found at for -275C>T, I62V, and IVS15, but not for Y402H (Table 3).

Genotype analysis showed no significant difference between the frequencies of Y402H genotypes in patients (TT, 79.8%; TC, 19.3%; CC, 0.9%) and controls (TT, 87.7%; TC, 11.8%; CC, 0.5%) (P=0.183). However, the genotype distributions of the other 3 SNPs were found to be significantly different in patients and controls (-275C>T, I62V, IVS15; P=0.008, <0.001, <0.001, respectively). Individuals homozygous with two risk alleles at -275C>T, I62V, and IVS15 were more likely to have AMD than those with no risk allele (OR: 2.295, 95% CI: 1.219-4.324; OR: 4.091, 95% CI: 1.963-8.526; OR: 3.430, 95% CI: 1.773-2.582), but individuals homozygous for C alleles at Y402H
showed no significant increase in risk (OR: 1.802, 95% CI: 0.111-29.155). Individuals with one risk allele (heterozygous) of the four SNPs did not show a significant increase in risk versus individuals with no risk allele. After adjusting for other risk factors, similar trends were observed (Table 3).
# Table 3. CFH SNP genotype and allele distributions among AMD cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele distribution</th>
<th>Genotype distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=114)</td>
<td>Controls (n=187)</td>
</tr>
<tr>
<td></td>
<td>χ²</td>
<td>p†</td>
</tr>
<tr>
<td>-275C&gt;T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(promoter)</td>
<td>T</td>
<td>127(55.7)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>101(44.3)</td>
</tr>
<tr>
<td>I62V</td>
<td>G</td>
<td>162(71.1)</td>
</tr>
<tr>
<td>(exon2)</td>
<td>A</td>
<td>68(28.9)</td>
</tr>
<tr>
<td>Y402H</td>
<td>C</td>
<td>24(10.5)</td>
</tr>
<tr>
<td>(exon9)</td>
<td>T</td>
<td>204(89.5)</td>
</tr>
<tr>
<td>IVS15</td>
<td>G</td>
<td>143(62.7)</td>
</tr>
<tr>
<td>(intron15)</td>
<td>A</td>
<td>85(37.3)</td>
</tr>
</tbody>
</table>

Abbreviation: AMD, age-related macular degeneration; CFH, complement factor H; CI, confidence interval; SNP, single nucleotide polymorphism; ORs, odds ratios.

* Indicates P value (chi-square test) for frequency across genotypes and alleles.

† Indicates P value as determined by logistic regression analysis.

‡ Odds ratio and 95% CIs are adjusted for age, gender, hypertension, hypercholesterolemia, and smoking status.

§ Odds ratios of individuals with 2 copies of the risk allele and individuals with no copy of the risk allele were compared.

π Odds ratios of individuals with one copy of the risk allele and individuals with no copy of the risk allele were compared.
3. Linkage disequilibrium and associations between haplotypes and AMD

The poor association between the Y402H variant and AMD was further confirmed by an analysis of pairwise linkage disequilibria (LD) among these SNPs in our cohort (Figure 1 and Table 4). Haplotype analysis based on the four SNPs predicted nine different haplotypes. Of these, 7 haplotypes with a frequency of over 1% were selected. Haplotype estimations in exudative AMD patients and controls identified two haplotypes as risk factors and one as a protective factor.

Specifically, two haplotypes, K1 (TGTG) and K7 (CGTG), were significantly associated with exudative AMD. These results are consistent with our SNP analysis results, shown in Table 3. The most frequent at-risk haplotype, K1 (TGTG), contained the major alleles of -275C>T, I62V, and IVS15, and occurred in 46.9% of patients and in 36.6% of controls. K1 was found to confer a 1.53 (95% CI: 1.095-2.137, P=0.013) fold increased likelihood of exudative AMD. Another minor but significant risk haplotype, K7 (CGTG) conferred a 3.917 (95% CI: 1.003-15.303, P=0.035) fold increased likelihood of exudative AMD. Homozygotes for the K1 accounted for 23.7% of cases and 11.8% of controls (OR: 2.328, 95% CI: 1.252-4.327, P=0.006), whereas no individual was found to be homozygous for K7. The protective haplotype, K2 (CATA), was found in 44.7% of controls and 26.3% of patients (OR: 0.443, 95% CI: 0.309-0.634, P<0.001) and homozygotes for this haplotype were present at frequencies of 21.9% and 9.6% in controls and AMD cases, respectively (OR: 0.380, 95% CI: 0.187-0.775, P=0.004).

Individuals heterozygous for one risk haplotype and another protective haplotype (K1/K2) showed a significantly lower risk of exudative AMD (OR: 0.506, 95% CI:
0.290-0.884, P=0.010). The protective haplotype K2 canceled out the effect of risk haplotype K1. Heterozygotes for two different risk haplotype (K1/K7) were found to have a 9.394 (95% CI: 1.082-81.524, P=0.023) fold increased likelihood having of exudative AMD (Table 4).

LD analysis showed extensive LD across an extended region of CFH. Three SNPs (I62V, Y402H, and IVS15) were virtually in complete LD, as were Y402H and IVS15 at exon9 and intron15, respectively (D’=1). As is made evident by figure 1, pairwise LD analysis showed I62V in high LD with Y402H (D’=1) and IVS15 (D’=0.896).
Figure 1. Linkage disequilibrium (LD) patterns for -275C>T, I62V, Y402H, and IVS15 in complement factor H (CFH). A set of the four informative single nucleotide polymorphisms (SNPs) in the CFH gene were analyzed for pairwise LD. R2 and D' values are shown. Squares shaded gray or black indicate significant LD between SNP pairs (HapAnalyzer ver. 1.0).
Table 4. *CFH* Haplotypes — Association analysis

<table>
<thead>
<tr>
<th>Haplotypes*</th>
<th>Haplotypes</th>
<th>Odds ratio (95%CI)</th>
<th>P</th>
<th>Frequency Cases (n=114)</th>
<th>Controls (n=187)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-275C&gt;T</td>
<td>I62V</td>
<td>Y402H</td>
<td>IVS15</td>
<td></td>
</tr>
<tr>
<td>K1†</td>
<td>T</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>1.530 (1.095-2.137)</td>
</tr>
<tr>
<td>K2‡</td>
<td>C</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>0.443 (0.309-0.634)</td>
</tr>
<tr>
<td>K3</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>1.636 (0.900-2.973)</td>
</tr>
<tr>
<td>K4</td>
<td>T</td>
<td>G</td>
<td>T</td>
<td>A</td>
<td>1.147 (0.614-2.143)</td>
</tr>
<tr>
<td>K5</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>A</td>
<td>1.417 (0.470-4.270)</td>
</tr>
<tr>
<td>K6</td>
<td>C</td>
<td>A</td>
<td>T</td>
<td>G</td>
<td>1.716 (0.369-3.749)</td>
</tr>
<tr>
<td>K7†</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>3.917 (1.003-15.303)</td>
</tr>
</tbody>
</table>

Diplotypes (Homozygous status)

| K1 / K1     | TGTG / TGTG | 2.328 (1.252-4.327) | 0.006 | 27 (23.7) | 22 (11.8) |
| K2 / K2     | CATA / CATA | 0.380 (0.187-0.775) | 0.004 | 11 (9.6) | 41 (21.9) |
| K7 / K7     | CGTG / CGTG | N/A                 | N/A   | 0 (0)     | 0 (0)     |

Diplotypes (Heterozygous status)

| K1 / K2     | TGTG / CATA | 0.506 (0.290-0.884) | 0.010 | 22 (19.3) | 60 (32.1) |
| K1 / K7     | TGTG / CGTG | 9.394 (1.082-81.527) | 0.023 | 5 (4.4) | 1 (0.5)   |
| K2 / K7     | CATA / CGTG | 0.823 (0.074-9.180) | 0.681 | 1 (0.9) | 2 (1.1)   |

Abbreviation: CFH, complement factor H; CI, confidence interval; N/A, not applicable.

* All haplotypes with a frequency of >1% are displayed.

† The dark grey highlight indicates risk haplotypes.

‡ The light grey highlight indicates the protective haplotypes.
Discussion

1. CFH gene associations in Koreans

Inflammation has been suggested to play a role in the pathogenesis of AMD, and of the molecules involved in the complement system, CFH (complement factor H) protein is known to critically regulate complement alternative pathway activation.20 In the present study, we focused on the associations between common 4 polymorphisms in CFH and AMD. Our results suggest that CFH polymorphisms are related with exudative AMD in Koreans. The Y402H variant showed a marginal association with AMD (P=0.071). However, the major alleles of the three other SNPs examined (-275C>T, I62V, IVS15) were found to significantly increase the risk of exudative AMD. The common haplotype K1, which possesses these SNPs (TGTG; with an estimated haplotype frequency of 46.9%), was found to be significantly associated with susceptibility to exudative AMD (OR: 1.530, 95% CI: 1.095-2.137). Moreover, homozygotes for this haplotype (K1/K1) were found to be at significant risk (OR: 2.328, 95% CI: 1.252-4.327).

-275C>T is located in the CFH promoter region, which regulates CFH transcription (e.g., nuclear factor-kappa B (NF-κB) responsive element).21-22 Therefore, it is possible that the function of this promoter is impaired by the presence of the T allele and that this leads to relatively lower factor H plasma levels.18 The exon2 I62V variant is located in SCR2 (short consensus repeats), which contains a regulatory domain for cofactor- and decay- accelerating activity, and a binding site for C3b.5,23 Furthermore, I62V is located in a predicted exon splice enhancer, and thus, this
polymorphism could cause splicing errors, and alter CFH function and AMD development.\textsuperscript{24}
2. The Y402H polymorphism and AMD risk

A number of studies in Caucasian populations have shown that Y402H SNP is significantly associated with AMD and that frequencies of the C allele range from 55 to 94% in AMD and from 34 to 46% in controls.\textsuperscript{5-14} In the present study, the frequency of the Y402H C allele was found to be lower in Koreans (10.5% in patients, 6.5% in controls) than in Caucasians, but to show minimal difference between patients and controls (P=0.071), and not to significantly increase the risk of exudative AMD (OR: 1.716, 95% CI: 0.950-3.100). Our results suggest that CFH polymorphisms are related to exudative AMD in Koreans, but that the influence of Y402H SNP is only marginal.

The exon9 Y402H variant lies within the SCR7 domain of CFH, which binds heparin and C-reactive protein. In previous studies, a high level association between this variant and the risk of AMD (by single SNP analyses) has focused attention on this variant. This attention is sustained despite observation of a stronger association between the disease and other nearby non-coding SNPs.\textsuperscript{25} Recent composite likelihood analysis of the association between AMD and the CFH gene suggested that the locus at the 5’ end of the CFH gene is also likely to be an important source of sequence variations.\textsuperscript{26}

Although the Y402H variant plays a major role in the etiology of AMD, it is unlikely to be the only major determinant of disease susceptibility. It may be that the CFH Y402H polymorphism is a susceptibility locus for the formation of soft drusen, but fewer than 30% of Caucasians that presenting with soft drusen eventually progress to an advanced form of AMD, thus, it appears that other variants are responsible for progression to advanced disease.\textsuperscript{27} This argument is supported by the observation that more than half of Caucasians possess at least one copy of the C allele, but the majority
do not develop AMD, and thus, other factors, both genetic and environmental, are likely
to determine risk. Moreover, the majority of Korean patients do not have Y402H, and
thus, other as yet unidentified genetic variants also probably promote disease
progression.
3. Ethnicity and CFH polymorphisms

The frequencies of CFH polymorphisms are obviously different in Caucasians and Asians. In the present study, we compared haplotype frequencies of two SNPs (I62V, Y402H) in Caucasians, Japanese, Chinese, and Koreans using the findings of the present and previous studies (Table 5).\textsuperscript{5,16,19} It was found that the haplotype frequencies in our AMD patients show the similarity to that in Japanese and Chinese. In Asian, haplotype H1 (GT) was more frequent in AMD patients and haplotype H2 (AT) in controls, whereas haplotype H3 (GC) is most common in Caucasians.

In terms of Y402H variant, Grassi et al. reported considerable ethnic variations and also suggested that other unidentified genetic factors evidently importantly contribute to the pathogenesis of AMD.\textsuperscript{15} The frequency of Y402H in Asians as determined by the International HapMap Project is much lower than in Caucasians, i.e., 8.1% in Japanese and 6.8% in Chinese versus more than 35% in Caucasians.\textsuperscript{15} In the present study, the frequency of the risk allele C was found to be 7.97%. Moreover, in agreement with our results, previous studies conducted in Japanese and Chinese populations concluded that the Y402H variant is not definitively associated with exudative AMD.\textsuperscript{16-19}

The only SNP reported in CFH promoter, -275C>T, was found to be unrelated to AMD in Caucasians.\textsuperscript{5,8} However, this association has been reported in Chinese,\textsuperscript{19} and we also detected a significant association in our study population. On the other hand, I62V has been reported to be associated with AMD in Caucasians,\textsuperscript{5,6} Chinese,\textsuperscript{19} and Japanese.\textsuperscript{17} Furthermore, Caucasians\textsuperscript{6} and Chinese\textsuperscript{19} homozygous for the C allele of IVS15 have been reported to have a higher risk of developing AMD.
It has been well established that the prevalence of AMD varies substantially between races. The prevalence of advanced AMD in Japanese is much lower than in Caucasians, and it has been suggested that this lower prevalence may be a consequence of the low frequency of Y402H variant in Asians. Moreover, the phenotypic spectrum of AMD among these different populations is also heterogeneous. For example, soft drusen, an inflammatory deposit containing complement factor H protein is less frequently observed in Japanese, and standard photodynamic therapy for wet AMD in Japanese patients is associated with greater angiographic and vision benefits than in Caucasians. However, no accurate epidemiologic study has been conducted in the Korean population, although it is generally assumed that disease phenotypes and treatment response are similar to those observed in the Japanese. We suspect that the low frequency of the C allele in Koreans is a reason for the low prevalence of AMD, rare features of uncontrolled soft drusen accumulation, and a better response. Therefore, we suggest that more detailed genotype-phenotype correlation studies be conducted in Asian populations.
**Table 5.** CFH Haplotype Comparisons of rs800292 (I62V) and rs1061170 (Y402H) in Koreans, Chinese, Japanese, and Caucasians

<table>
<thead>
<tr>
<th></th>
<th>Koreans</th>
<th>Chinese *</th>
<th>Japanese †</th>
<th>Caucasians ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>H1 (GT)</td>
<td>0.605</td>
<td>0.463</td>
<td>0.69</td>
<td>0.58</td>
</tr>
<tr>
<td>H2 (AT)</td>
<td>0.289</td>
<td>0.473</td>
<td>0.25</td>
<td>0.38</td>
</tr>
<tr>
<td>H3 (GC)</td>
<td>0.105</td>
<td>0.064</td>
<td>0.058</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Abbreviation: CFH, complement factor H; N/A, not available.

4. Potential Weaknesses

The main study limitation concerns its low sample size. To statistically distinguish between cases and controls in terms of Y402H frequencies, 182 patients and 182 controls should have been recruited, which would have provided sufficient statistical power to detect differences of ca. 4% (\(\alpha=0.05\), statistical power=0.8). Thus, the present study is limited in terms of detecting significant differences. However, it was difficult to calculate appropriate sample sizes based on limited knowledge of the proportion of individuals who carry the risk allele (Y402H) in the Korean population. More comprehensive analysis of variations at this locus is necessary in a larger population.
Conclusion

The purpose of the present study was to investigate the association of *CFH* gene in Korean patients with exudative AMD. In our Korean cohort, haplotype analysis indicated that the *CFH* gene meaningfully contributes to exudative AMD. Moreover, the Y402H variant was found to be much less frequent in our Korean cohort than has been observed in Caucasians, nevertheless, it was found to be marginally associated with AMD.

Further studies in different ethnic populations widen our understanding of the role of the *CFH* polymorphisms with respect to susceptibility to AMD. It is hoped that, in the future, at-risk populations can be identified decades before likely clinical AMD manifestation, as this would allow the individual to make lifestyle and nutritional modifications, and facilitate the initiation of yet to be discovered therapies.
References


