# Association between Complement Factor H Gene Polymorphisms and Neovascular Age-Related Macular Degeneration in Koreans

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**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 patients with exudative AMD ($n = 114$) and control subjects ($n = 187$). The sole criterion for exudative AMD was the presence of choroidal neovascularization. Four single-nucleotide polymorphisms (SNPs: $-275C>T$, I62V, Y402H, and IVS15) located in promoter, exon 2, exon 9, and intron 15 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, the difference between the frequencies of Y402H in cases and control subjects 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 control subjects, 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 ($P < 0.001$).

**CONCLUSIONS.** 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 found to be only marginally associated with exudative AMD with low frequency, whereas three adjacent SNPs in the *CFH* gene were significantly associated with AMD in Koreans. (*Invest Ophthalmol Vis Sci.* 2008;49:2071–2076) DOI:10.1167/iovs.07-1195

Age-related macular degeneration (AMD) is the primary cause of severe vision 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 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 1, area q31, has been reported to be a risk factor for AMD in North America and Europe.[5–14] However, the majority of these previous studies enrolled 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.[16–19] 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. To the best of our knowledge, this is the first study to investigate the association between genetic factors and exudative AMD in Korean patients. Here, we evaluated the relationships between four common polymorphisms in the *CFH* gene—namely, $-275C>T$, I62V, Y402H, and IVS15 (SNP ID: rs3753394, rs800292, rs1061170, and rs1329428) and AMD in Koreans.

## METHODS

### 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.

TABLE 1. Baseline Characteristics of the Study Subjects*

|  | Cases (n = 114) | Controls (n = 187) | All Subjects (n = 301) | P |
|---|---|---|---|---|
| Ages (y)† | 68.25 ± 8.806 | 66.83 ± 7.794 | 67.37 ± 8.207 | P = 0.148 |
| Sex, n (%) |  |  |  |  |
| Male | 47 (41.2) | 94 (50.3) | 141 (46.8) | P = 0.127 |
| Female | 67 (58.8) | 93 (49.7) | 160 (53.2) |  |

* Cases and controls were matched for age and sex.
† Mean ± SD.

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 by using the same protocol. Inclusion criteria were as follows: (1) age >50 years; (2) a diagnosed 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, angioid 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 50 years of age. In addition, the control subjects had no known family history of AMD. Fundus photography was performed routinely on all control subjects.

## Genotyping

Peripheral venous blood was collected from all subjects into 5-mL EDTA tubes. Genomic DNA was extracted from leukocytes by using commercially available kits (QIAmpDNA; Qiagen, Valencia, CA), and polymorphic sites were amplified by PCR with specific primers. PCR products were used as the templates for direct DNA sequencing (Big Dye Terminator cycle sequencing kits; Applied Biosystems, Inc. [ABI], Foster City, CA) on an automated sequencer (model 3730; ABI).

## Statistics

Allele and genotype frequencies were estimated by using the allele-counting method. Hardy-Weinberg estimates for genotypes and estimated haplotype frequencies were calculated with the HapAnalyzer (ver. 1.0; a minimum haplotype analysis system for association studies provided in the public domain by the National Genome Research Institute, Seoul, Korea; http://hap.ngri.go.kr/right.html).

Numerical data were analyzed with 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: age (50–59 years, 0; 60–69 years, 1; and ≥70 years, 2), gender (male, 0; female, 1), smoking status (non-smoking, 0; <20 pack-years, 1; ≥20 pack-years, 2), and hypercholesterolemia (<200, 0; ≥ 200, 1).

The allele and genotype frequencies of cases and control subjects were compared by $\chi^2$ analysis. $\chi^2$ 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).

Pair-wise linkage disequilibrium (LD; D') estimations between SNPs at the *CFH* locus and EM-based haplotype association analysis were performed using the HapAnalyzer software. All haplotypes with a frequency of >1% were selected. ORs for the cases and control subjects were calculated, and 95% CIs were calculated. Homozygotes and heterozygotes for these haplotypes among cases and control subjects were compared by $\chi^2$ test.

Statistical analysis was performed with commercial software (SPSS for Windows, ver. 12.0; SPSS Inc., Chicago, IL). The criterion for statistical significance was $P \leq 0.05$.

## RESULTS

### Participants

In total, 114 patients with exudative AMD and 187 control subjects participated in the study. The baseline characteristics of patients and control subjects are shown in Table 1. The

TABLE 2. Description of Variables

|  | Cases (n = 114) | Controls (n = 187) | P* | P | Odds Ratio (95% CI)† |
|---|---|---|---|---|---|
| Age (y) |  |  |  |  |  |
| 50–59 | 15 (13.2) | 33 (17.6) |  |  | 1.000 (reference) |
| 60–69 | 48 (42.1) | 99 (52.9) |  | 0.857 | 1.067 (0.529–2.150) |
| ≥70 | 51 (44.7) | 55 (29.4) | 0.027 | 0.052 | 2.040 (0.993–4.189) |
| Sex |  |  |  |  |  |
| Male | 47 (41.2) | 94 (50.3) |  |  | 1.000 (reference) |
| Female | 67 (58.8) | 93 (49.7) | 0.128 | 0.128 | 1.441 (0.900–2.306) |
| Hypertension‡ |  |  |  |  |  |
| No | 39 (34.2) | 79 (43.2) |  |  | 1.000 (reference) |
| Yes | 75 (65.8) | 104 (56.8) | 0.126 | 0.126 | 1.461 (0.899–2.373) |
| Cholesterol (mg/dL) |  |  |  |  |  |
| <200 | 33 (28.9) | 71 (38.0) |  |  | 1.000 (reference) |
| ≥200 | 81 (71.1) | 116 (62.0) | 0.111 | 0.111 | 1.502 (0.910–2.480) |
| Cigarette smoking§ |  |  |  |  |  |
| Never | 60 (52.6) | 128 (68.4) |  |  | 1.000 (reference) |
| <20 | 24 (21.1) | 30 (16.0) |  | 0.090 | 1.707 (0.920–3.167) |
| ≥20 | 30 (26.3) | 29 (15.5) | 0.019 | 0.009 | 2.207 (1.217–4.003) |

* $\chi^2$ test for frequency.
† ORs and 95% CIs were determined by logistic regression analysis.
‡ Sample sizes sum to slightly less than the total number of cases and controls because a small amount of covariate data were unavailable.
§ Pack years.

mean ages ($P = 0.148$) and gender distributions ($P = 0.127$) of the patients and control subjects 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 sex ($P = 0.128$), hypercholesterolemia ($P = 0.111$), or hypertension ($P = 0.126$). Hypercholesterolemia and hypertension tended to be more prevalent in patients than in control subjects (Table 2).

## Association between the Four SNPs and AMD

The genotype frequency distributions of patients and control subjects 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 control subjects. The different Y402H frequencies of cases and control subjects did not reach statistical significance ($P = 0.071$; 10.5% in exudative AMD cases, and 6.5% in control subjects). 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 cases and control subjects. The major alleles of the three SNPs (T for −275C>T, G for I62V, and G for IVS15) conferred 1.576-fold (95% CI: 1.131–2.195, $P = 0.007$), 2.205-fold (95% CI: 1.553–3.132, $P < 0.001$), and 1.997-fold (95% CI: 1.426–2.797, $P < 0.001$) increases in the likelihood of exudative AMD, respectively. After adjusting for known risk factors, significant associations were found 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 control subjects (TT, 87.7%; TC, 11.8%; CC, 0.5%; $P = 0.183$). However, the genotype distributions of the other three SNPs were found to be significantly different in cases and control subjects (−275C>T, I62V, and IVS15; $P = 0.008$, < 0.001, and < 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 adjustment for other risk factors, similar trends were observed (Table 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 pair-wise LD among these SNPs in our cohort (Table 4, Fig. 1). Haplotype analysis based on the four SNPs predicted nine different haplotypes. Of these, seven haplotypes with a frequency of >1% were selected.

Haplotype estimations in patients with exudative AMD and control subjects 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 control subjects. K1 was found to confer a 1.53-fold (95% CI: 1.095–2.137, $P = 0.013$) increased likeli-

**TABLE 3.** *CFH* SNP Genotype and Allele Distributions among AMD Cases and Controls

| SNP (Position) | | Allele Distribution | | | | | | Genotype Distribution | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | Cases (n = 114) | Controls (n = 187) | P* | P† | OR (95% CI)‡ | | Cases (n = 114) | Controls (n = 187) | P* | P† | OR (95% CI)‡ |
| −275C>T (Promoter) | T | 127 (55.7) | 166 (44.3) | 0.007 | 0.018 | 1.524 (1.074–2.163) | TT | 41 (36.0) | 37 (19.8) | 0.008 | 0.025 | 2.149 (1.100–4.114)§ |
| | C | 101 (44.3) | 208 (56.6) | | | | TC | 45 (39.5) | 92 (49.2) | | 0.539 | 0.823 (0.443–1.531)‖ |
| | | | | | | | CC | 28 (24.6) | 58 (31.0) | | | 1.000 (reference) |
| 162V (Exon2) | G | 162 (71.1) | 197 (52.6) | <0.001 | <0.001 | 2.172 (1.499–3.146) | GG | 60 (52.6) | 55 (29.4) | <0.001 | <0.001 | 3.957 (1.835–8.535)§ |
| | A | 68 (28.9) | 177 (47.3) | | | | GA | 42 (36.8) | 87 (46.5) | | 0.186 | 1.677 (0.779–3.609)‖ |
| | | | | | | | AA | 12 (10.5) | 45 (24.1) | | | 1.000 (reference) |
| Y402H (Exon9) | C | 24 (10.5) | 24 (6.5) | 0.071 | 0.064 | 1.827 (0.965–3.460) | CC | 1 (0.9) | 1 (0.5) | 0.183 | 0.545 | 2.514 (0.128–49.552)§ |
| | T | 204 (89.5) | 350 (93.5) | | | | CT | 22 (19.3) | 22 (11.8) | | 0.076 | 1.876 (0.937–3.758)‖ |
| | | | | | | | TT | 91 (79.8) | 164 (87.7) | | | 1.000 (reference) |
| IVS15 (Intron15) | G | 143 (62.7) | 171 (45.7) | <0.001 | <0.001 | 1.943 (1.363–2.769) | GG | 49 (43.0) | 40 (21.4) | <0.001 | 0.001 | 3.298 (1.648–6.563)§ |
| | A | 85 (37.3) | 203 (54.3) | | | | GA | 45 (39.5) | 91 (48.7) | | 0.298 | 1.412 (0.738–2.705)‖ |
| | | | | | | | AA | 20 (17.5) | 56 (29.9) | | | 1.000 (reference) |

* $\chi^2$ test for frequency across genotypes and alleles.
† By logistic regression analysis.
‡ Adjusted for age, gender, hypertension, hypercholesterolemia, and smoking status.
§ Individuals with two copies of the risk allele and individuals with no copy of the risk allele were compared.
‖ Individuals with one copy of the risk allele and individuals with no copy of the risk allele were compared.

**TABLE 4.** *CFH* Haplotypes: Association Analysis

| | −275C>T | I62V | Y402H | IVS15 | Odds Ratio (95% CI) | *P* | Frequency Cases (*n* = 114) | Frequency Controls (*n* = 187) |
|---|---|---|---|---|---|---|---|---|
| Haplotypes* | | | | | | | | |
| K1† | T | G | T | G | 1.530 (1.095–2.137) | 0.013 | 107 (46.9) | 137 (36.6) |
| K2‡ | C | A | T | A | 0.443 (0.309–0.634) | <0.001 | 60 (26.3) | 167 (44.7) |
| K3 | C | G | C | G | 1.636 (0.900–2.973) | 0.103 | 23 (10.1) | 24 (6.4) |
| K4 | T | G | T | A | 1.147 (0.614–2.143) | 0.666 | 18 (7.8) | 26 (6.95) |
| K5 | C | G | T | A | 1.417 (0.470–4.270) | 0.534 | 6 (2.63) | 7 (1.87) |
| K6 | C | A | T | G | 1.716 (0.369–3.749) | 0.784 | 5 (2.19) | 7 (1.87) |
| K7† | C | G | T | G | 3.917 (1.003–15.303) | 0.035 | 7 (3.1) | 3 (0.8) |
| Diplotypes (homozygous status) | | | | | | | | |
| K1/K1† | | T G T G/T G T G | | | 2.328 (1.252–4.327) | 0.006 | 27 (23.7) | 22 (11.8) |
| K2/K2‡ | | C A T A/C A T A | | | 0.380 (0.187–0.775) | 0.004 | 11 (9.6) | 41 (21.9) |
| K7/K7 | | C G T G/C G T G | | | N/A | N/A | 0 (0) | 0 (0) |
| Diplotypes (heterozygous status) | | | | | | | | |
| K1/K2‡ | | T G T G/C A T A | | | 0.506 (0.290–0.884) | 0.010 | 22 (19.3) | 60 (32.1) |
| K1/K7† | | T G T G/C G T G | | | 9.394 (1.082–81.524) | 0.023 | 5 (4.4) | 1 (0.5) |
| K2/K7 | | C A T A/C G T G | | | 0.823 (0.074–9.180) | 0.681 | 1 (0.9) | 2 (1.1) |

\* All haplotypes with a frequency of >1% are displayed.
† Risk haplotypes.
‡ Protective haplotypes.

hood of exudative AMD. Another minor but significant risk haplotype, K7 (CGTG) conferred a 3.917-fold (95% CI: 1.003–15.303, *P* = 0.035) increased likelihood of exudative AMD. Homozygotes for K1 accounted for 23.7% of cases and 11.8% of control subjects (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 control subjects 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 control

subjects 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-fold (95% CI: 1.082–81.524, *P* = 0.023) 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 exon 9 and intron 15, respectively (D' = 1). As is evident in Figure 1, pair-wise LD analysis showed I62V in high LD with Y402H (D' = 1) and IVS15 (D' = 0.896).

## DISCUSSION

### 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 as a critical regulator of the complement alternative pathway activation.[20] In the present study, we focused on the associations between the common four polymorphisms in *CFH* and AMD. Our results suggest that the *CFH* polymorphisms are associated 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 increase the risk of exudative AMD significantly. 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 [NF]-κB re-
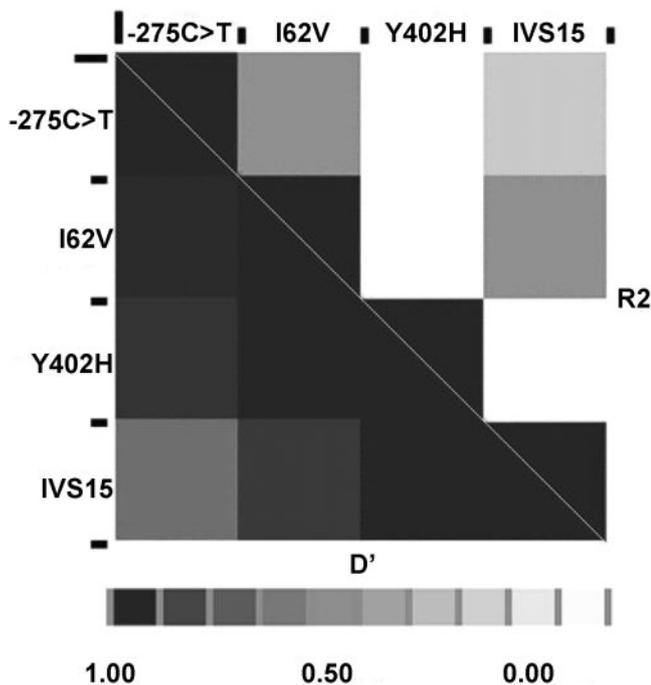


**FIGURE 1.** LD patterns for −275C>T, I62V, Y402H, and IVS15 in *CFH*. A set of the four informative SNPs in the *CFH* gene were analyzed for pair-wise LD. R2 and D' values are shown. *Gray* or *black squares*: significant LD between SNP pairs (HapAnalyzer ver. 1.0).

**TABLE 5.** *CFH* Haplotype Comparisons of rs800292 (I62V) and rs1061170 (Y402H) in Koreans, Chinese, Japanese, and Caucasians

| | Koreans | | Chinese* | | Japanese† | | Caucasians‡ | |
|---|---|---|---|---|---|---|---|---|
| | Cases | Controls | Cases | Controls | Cases | Controls | Cases | Controls |
| H1 (GT) | 0.605 | 0.463 | 0.69 | 0.58 | 0.62 | 0.57 | N/A | N/A |
| H2 (AT) | 0.289 | 0.473 | 0.25 | 0.38 | 0.30 | 0.40 | 0.12 | 0.21 |
| H3 (GC) | 0.105 | 0.064 | 0.058 | 0.039 | 0.08 | 0.03 | 0.50 | 0.29 |

\* Chinese: Chen et al.[19]
† Japanese: Gotoh et al.[16]
‡ Caucasians: Hageman et al.[5]

sponsive 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, 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.[24]

### The Y402H Polymorphism and AMD Risk

Several 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 control subjects.[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 control subjects) than in Caucasians, but to show minimal difference between patients and control subjects ($P = 0.071$) and not to increase significantly 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 exon 9 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 noncoding SNPs.[25] A 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.[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 present with soft drusen eventually progress to an advanced form of AMD; thus, it appears that other variants are responsible for progression to advanced disease.[27] This argument is supported by the observation that more than half of Caucasians possess at least one copy of the C allele, but most do not develop AMD, and thus, other factors, both genetic and environmental, are likely to determine risk.[12] Moreover, most Korean patients do not have Y402H, and thus, other as yet unidentified genetic variants also probably promote disease progression.

### 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).[5,16,19] It was found that the haplotype frequencies in our patients with AMD show the similarity to that in Japanese and Chinese. In Asian, haplotype H1 (GT) was more frequent in patients with AMD and haplotype H2 (AT) in control subjects, whereas haplotype H3 (GC) is most common in Caucasians.

In terms of Y402H variant, Grassi et al.[15] reported considerable ethnic variations and also suggested that other unidentified genetic factors evidently importantly contribute to the pathogenesis of AMD. 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).[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.[16-19]

The only SNP reported in the *CFH* promoter −275C>T was found to be unrelated to AMD in Caucasians.[5,8] However, this association has been reported in China,[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,[5-6] Chinese,[19] and Japanese.[17] Furthermore, Caucasians[6] and Chinese[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.[28,29] The prevalence of advanced AMD in Japanese is much lower than in Caucasians,[29] and it has been suggested that this lower prevalence may be a consequence of the low frequency of Y402H variant in Asians.[11] Moreover, the phenotypic spectrum of AMD among these different populations is also heterogeneous. For example, soft drusen, an inflammatory deposit containing CFH protein is less frequently observed in the Japanese,[29-31] and standard photodynamic therapy for wet AMD in Japanese patients is associated with greater angiographic and vision benefits than in Caucasians.[31] 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, and suggest that more detailed genotype–phenotype correlation studies be conducted in Asian populations.

### Potential Weaknesses

The main study limitation is its low sample size. To distinguish statistically the Y402H frequency in cases and control subjects, 182 patients and 182 control subjects should have been recruited, which would have provided sufficient statistical power to detect differences of ~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

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.

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