Association of the Y402H Polymorphism in Complement Factor H Gene and Neovascular Age-Related Macular Degeneration in Chinese Patients

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PURPOSE. Age-related macular degeneration (AMD), with its complex traits and multiple risk factors, is the leading cause of blindness in the elderly. A strong association between a coding variant, Y402H, in the complement factor H gene (CFH) and AMD has been recently identified in white patients. This study was conducted to investigate the association between the Y402H polymorphism in CFH and neovascular AMD in Chinese patients.

METHODS. One hundred sixty-three Chinese patients with neovascular AMD and 232 age-matched healthy controls were enrolled in the study. Genomic DNA from white blood cells was extracted. The Y402H polymorphism in CFH, with the substitution of T to C at nucleotide position 1277 in exon 9, was determined by polymerase chain reaction–restriction fragment length polymorphism analysis. The association between the genetic polymorphism and the disease was examined by χ² test and logistic regression.

RESULTS. The frequency of the risk allele, 1277C, was 11.3% in AMD patients compared with 2.8% in controls (P < 0.00001). Genotype frequency differed significantly between the two groups (1277TT 81.0%, 1277TC 15.3%, and 1277CC 3.7% in the AMD group; 1277TT 94.4%, 1277TC 5.6%, and 1277CC 0% in the control group; P < 0.0001). The 1277C allele significantly increased the risk for neovascular AMD and had an odds ratio of 4.4 (95% confidence interval [95% CI], 2.3–8.5; P < 0.00001).

CONCLUSIONS. The allele frequency of Y402H polymorphism in CFH has an ethnic variation, with much lower 1277C frequency in Chinese than in white patients. Despite this, the polymorphism is significantly associated with neovascular AMD in the Chinese population. ( Invest Ophthalmol Vis Sci. 2006;47:3242–3246) DOI:10.1167/iovs.05-1532

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Age-related macular degeneration (AMD) is one of the leading causes of blindness among patients 50 years and older, not only in Western countries1–3 but also in Taiwan.4 The clinical hallmark of AMD is the appearance of drusen, localized deposits lying between the basement membrane of the retinal pigment epithelium (RPE) and Bruch membrane.4 The central vision loss of AMD is attributable to degenerative and neovascular changes at the macula.4 Despite the high socioeconomic burden it causes, the pathogenesis of AMD remains unclear. The contemporary view is that AMD is a complex disorder stemming from the interaction of multiple genetic and environmental risk factors.5

The US twin study of AMD found that genetic factors play a substantial role in the etiology of the disease.6 Linkage analyses have reported several susceptible loci, including the 1q31, 10q26, 16p12, and 17q25 regions.7–9 Among these regions, the locus on chromosome 1q provides the most consistent evidence linked to AMD. Klein et al.9 were the first to map the susceptible locus to chromosome 1q25-q31 in a large family with AMD. Many subsequent large-scale genetic studies also support this linkage result.10–13 Recently, three independent studies identified a polymorphism with a coding variant, Y402H, in the complement factor H gene (CFH) located on chromosome 1q31 that significantly increases the risk for AMD.14–16 The T → C transition at nucleotide position 1277 in exon 9 of CFH results in a tyrosine → histidine substitution at codon 402 of the protein.17 Allelic association studies revealed a peak association between the Y402H variant and AMD. Many other study groups replicated the study, and all showed consistent results of significant association between the Y402H polymorphism in CFH and AMD.18–25

Thus far, all the association studies of the Y402H polymorphism and AMD have been performed in white patients.14–16,18–25 Further studies in different ethnic populations would facilitate understanding of the role of the risk allele in the susceptibility of human subjects to AMD. Moreover, there may be an ethnic difference in the allele frequencies of this polymorphism. In this study, we aimed to investigate the allele frequencies of the Y402H polymorphism and its association with neovascular AMD in the Chinese population.

METHODS

Patients

This was a hospital-based, case-control association study undertaken in a Chinese population. Patients with AMD were compared with unrelated control subjects. AMD patients were recruited from the Retinal Clinic of the Department of Ophthalmology, Taipei Veterans General Hospital, Taiwan. All study participants underwent complete ophthalmoscopic examination, including visual acuity measurement, slit lamp biomicroscopy, dilated fundus examination, color fundus photography, and fluorescein angiography of the macular area. Recruited patients were older than 55 and had neovascular AMD in at least one eye.
Neovascular AMD was defined by ophthalmoscopic and fluorescein angiographic findings of classic or occult choroidal neovascular membranes, serous or hemorrhagic RPE detachments, and fibrovascular disciform scar in reference to the International Classification of Age-Related Maculopathy and Macular Degeneration. Control subjects—age-matched healthy persons without visual impairment—were recruited from the outpatient department during routine ophthalmic examination. They underwent dilated fundus examination to confirm the absence of any type of drusen, geographic atrophy, neovascular AMD, or other retinal disorder. To eliminate the confounding effect of systemic disease, patients who had renal function impairment, hematologic disease, benign or malignant tumors, or diabetes mellitus were also excluded from this study. Informed consent was obtained from all subjects by way of a consent form approved by the Institutional Review Board for Human Research of Taipei Veterans General Hospital. All procedures adhered to the tenets of the Declaration of Helsinki.

Genotyping

Genotyping was carried out in random order by an experienced technician who was masked to the disease status of the samples. An aliquot of 5 mL venous blood from each subject was withdrawn and collected in an EDTA-containing tube. Genomic DNA was extracted by serial phenol/chloroform extraction and ethanol precipitation. The polymorphism of CFH at the nucleotide position 1277 was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. An aliquot of 100 ng genomic DNA was added to a 50-μL PCR mixture containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.1% Triton X-100, 2 mM MgCl2, 0.2 mM each dNTP, 100 pmol each primer, and 1 U Taq DNA polymerase (Biotools; B & M Laboratories, Madrid, Spain). PCR was performed with the use of 5′-TCA TTG TTA TGG TCC TTA GGA AA-3′ as the forward primer and 5′-TTA AGA AGA CAT GAA CAT GCT AGG-3′ as the reverse primer. The thermal profile consisted of 30 cycles of denaturation at 94°C for 40 seconds, annealing at 57°C for 40 seconds, and polymerization at 72°C for 40 seconds, preceded by an initial denaturation step at 94°C for 5 minutes and followed by a terminal extension at 72°C for 5 minutes. The amplified DNA fragment measured 241 bp and contained the 1277 polymorphic site. After PCR, 10 μL product was subjected to digestion with 2.5 U Tsp509I (New England Biolabs, Ipswich, MA) in a 15-μL reaction mixture according to the manufacturer’s instructions. The presence of T at nucleotide position 1277 created a recognition site for Tsp509I, which led to digestion of the 241-bp PCR product into two DNA fragments of 60 bp and 181 bp (Fig. 1). Digested PCR products were separated by electrophoresis on a piece of 2% agarose gel, followed by staining with ethidium bromide. To verify the PCR-RFLP results, we sequenced two batches of PCR product of each genotype (1277TT, 1277TC, 1277CC) from each group. Sequencing results were consistent with the PCR-RFLP results in all cases.

Statistical Analysis

Statistical analysis was performed with SPSS, version 11 (SPSS Inc., Chicago, IL). Categorical data between the two groups were analyzed and compared with the χ2 test. Hardy-Weinberg equilibrium for genotypes distribution in the two groups was analyzed by the χ2 test. Numerical data were examined by Student t test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated after adjustment for age and sex by logistic regression. P < 0.05 was considered statistically significant.

Results

In total, 163 patients with neovascular AMD and 232 healthy controls were enrolled in this study. Mean ages of the AMD patients and the healthy controls were 76.4 ± 7.2 and 75.9 ± 7.4 years, respectively (P = 0.54, Student t test). Percentages of men in the two groups were 87.1% and 78.0%, respectively (P = 0.03, χ2 test). Allele frequencies for 1277C were 11.3% in AMD patients and 2.8% in healthy controls (P < 0.0001, χ2 test; Table 1). The 1277C allele contributed to a 4.4-fold (95% CI, 2.4–8.5; P < 0.0001) increased risk for neovascular AMD in Chinese patients. The distribution of genotypes among AMD patients was significantly different from that among healthy subjects (P < 0.0001, χ2 test; Table 2). Patients who carried at least one copy of the 1277C allele were at 3.9-fold greater risk for neovascular AMD (95% CI, 2.0–7.8; P = 0.0001) after adjustment for age and sex. However, the susceptibility risk for neovascular AMD in those who carried two copies of the 1277C allele compared with those who carried no more than one 1277C allele could not be estimated because too few subjects had the homozygous 1277CC genotype in the population studied (six in the AMD group, none in the control group). The genotype distribution of the Y402H polymorphism reached Hardy-Weinberg equilibrium in the control group but not in the AMD group (χ2 = 0.19, control group; χ2 = 9.22, AMD group).

Discussion

Our study demonstrated that the Y402H polymorphism in CFH was significantly associated with susceptibility to neovascular AMD in Chinese patients. The 1277C allele was associated with a 4.4-fold increased risk for neovascular AMD. Participants who carried at least one 1277C allele were at 3.9-fold higher risk for neovascular AMD than participants who carried no 1277C allele after adjustment for the factors of age and sex.

The high proportion of men in this study was attributed to the fact that the hospital was a veterans hospital. However, the factor of unbalanced sex ratio had been taken into account when performing logistic regression to calculate the ORs. Because some mutations and polymorphisms in CFH are associated with hemolytic uremic syndrome, we excluded all patients with renal function impairment or hematologic disease.
Few epidemiologic data of the prevalence of AMD are available in Taiwan. In a nationwide survey supported by the Bureau of Health Promotion, the crude estimation of the prevalence of AMD among subjects older than 65 is 2.9% in Taiwan.²⁵ Given that the study does not specify the subtype of AMD, comparison with the data of white patients is not feasible. However, the prevalence of AMD in the Taiwanese population is probably lower than it is in white persons (ranges, 10.3%–22.5% in early AMD and 2.4%–5.6% in advanced AMD in the white population).²⁶–²⁸ In China, the prevalence of AMD varies considerably among ethnic groups, with 6.4% in Han, 11.3% in Uighur, and 15.6% in Tibetans.²⁹ Although detailed and direct comparison among epidemiologic studies could not be performed because of different study designs, age groups, and definitions of AMD, the prevalence of AMD seems to vary considerably around the world by ethnic group.³⁰ This ethnic difference may reflect complex interactions between genetic and environmental factors in the pathogenesis of AMD.

The association between the Y402H polymorphism and neovascular AMD is consistently reported in Northern American and European populations.¹⁶,¹⁹–²³ Haines et al.¹⁶ reported odds ratios of 3.4 and 5.6 for the CT and CC genotypes, respectively, in Northern American patients with neovascular AMD. Odds ratios revealed by Souied et al.²¹ in the French population were 3.0 and 6.93 in neovascular AMD patients with the CT and CC genotypes, respectively. Sepp et al.²⁵ found odds ratios of 2.7 and 5.1 for the CT and CC genotypes, respectively, in patients with neovascular AMD in the United Kingdom. Magnusson et al.²² showed that the 1277C allele conferred ORs of 2.32 and 2.17 in comparisons of patients with neovascular AMD and healthy persons in Iceland and Utah. Conley et al.²⁰ found that the 1277C allele increased the risk for neovascular AMD, with an OR of 3.46 in white persons. Consistent with these results, our study found that the 1277C allele contributed to neovascular AMD with an OR of 4.4 in Chinese persons. However, the 1277C frequency is much lower in Chinese than in other populations. In our study, the 1277C frequency was low in the AMD and the control groups (neovascular AMD, 11.3%; controls, 2.8%), whereas in Northern American and European populations, the risk allele frequency exceeds the non–risk allele frequency in the AMD group (neovascular AMD, 55.9%–59.0%; controls, 35.4%–39.9%).²¹–²³ Despite this low frequency, the 1277C allele significantly increased the risk for neovascular AMD in Chinese (OR, 4.4). Moreover, our study showed that more homozygous persons carried the risk allele in the AMD group than in the control group (6 versus 0 persons). The excess in homozygosity of the risk allele might have resulted in deviation from Hardy-Weinberg equilibrium in the AMD group, whereas the control group remained in equilibrium. The difference in

### Table 1. Allele Frequency and Odds Ratio for the Y402H Polymorphism in Neovascular AMD Patients and Controls

<table>
<thead>
<tr>
<th>Alleles</th>
<th>AMD Group (n = 163)</th>
<th>Control Group (n = 232)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1277T (%)</td>
<td>37 (11.3)</td>
<td>13 (2.8)</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>1277T (%)</td>
<td>289 (88.7)</td>
<td>451 (97.2)</td>
<td>—</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>—</td>
<td>4.4 (2.3–8.5)</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Data are expressed as the number of subjects (% of the entire group).

* P value for the difference in allele frequency between the two groups by χ² test.

† OR and 95% CI for the 1277C allele was calculated by logistic regression with the 1277T allele as the reference group, adjusted for age and sex.

### Table 2. Genotype Distribution and Dominant Odds Ratio for the Y402H Polymorphism in Neovascular AMD Patients and Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>AMD Group (n = 163)</th>
<th>Control Group (n = 232)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1277CC (%)</td>
<td>6 (3.7)</td>
<td>0 (0)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>1277CT (%)</td>
<td>25 (15.3)</td>
<td>13 (5.6)</td>
<td>—</td>
</tr>
<tr>
<td>1277TT (%)</td>
<td>132 (81.0)</td>
<td>219 (94.4)</td>
<td>—</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>3.9 (2.0–7.8)</td>
<td>—</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as the number of subjects (% of the entire group).

* P value for the difference in genotype distribution between the two groups by χ² test.

† Dominant OR and 95% CI for each participant with at least one copy of the 1277C allele was calculated by logistic regression with the 1277TT genotype as the reference group, adjusted for age and sex.
ment cascade. Intense CFH immunofluorescence is also noted in the sub-RPE space and around the choroidal capillaries, especially in the macular region. Moreover, abundant transcripts of CFH are also identified in the RPE and choroid, indicating local production of this regulatory protein. All these findings support the hypothesis that local inflammation and aberrant complement regulation in the outer retina contribute to the pathogenesis of AMD. In addition, risk factors for AMD, including cigarette smoking, hypertension, and obesity, are associated with increased serum CRP or decreased serum CFH levels. Furthermore, drusen also developed in patients with membranoproliferative glomerulonephritis type II (MPGNII), a rare renal disease associated with CFH deficiency. The disease can be caused by mutations of CFH. Additionally, patients with MPGNII may harbor the CFH at-risk haplotype, as do patients with AMD. Apart from its association with advanced AMD, the Y402H polymorphism in CFH also increases the risk for soft drusen, an important precursor of neovascular AMD.

In conclusion, our data demonstrated a significant association between the Y402H polymorphism and neovascular AMD in Chinese patients. Although the risk allele frequency is low in this population, the 1277C allele increases the risk for neovascular AMD with an OR of 4.4. Those who carried the homoygous 1277CC genotype had a high susceptibility to neovascular AMD, whereas none of the healthy controls in this study carried this genotype. However, because most AMD patients do not carry the risk allele, additional important genetic or environmental factors may contribute to the pathogenesis of AMD. The genetic influence of Y402H polymorphism on AMD seems to be different between Chinese and white populations, and further studies are warranted to fully elucidate the mechanism.

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References


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**ERRATUM**


The correct spelling of the fifth author’s name is Abhiram S. Vilupuru.