Coding and Noncoding Variants in the *CFH* Gene and Cigarette Smoking Influence the Risk of Age-Related Macular Degeneration in a Japanese Population

Keisuke Mori,[1] *Peter L. Gehlbach,*[2] *Sho Kabasawa,*[1] *Izumi Kawasaki,*[1] *Masataka Oosaki,*[3] *Hiroyuki Iizuka,*[4] *Shigehiro Katayama,*[3] *Takuya Awata,*[3,4] *and Shin Yoneya*[1]

Purpose. Ethnic variation has been reported in age-related macular degeneration (AMD)–associated Y402H polymorphism in complement factor H (*CFH*). This variation is evident in the Japanese population. Recently a strong association between a novel single-nucleotide polymorphism (SNP; rs1410996) in the *CFH* gene and AMD has been identified in Caucasian patients. The present study was undertaken to investigate whether four coding and noncoding variants of the *CFH* gene, including rs1410996, are associated with AMD in native, unrelated Japanese patients.

Methods. A total of 188 patients with AMD and 139 control subjects without AMD were recruited for the study. Four SNPs (rs800292, rs1061170, rs1410996, and rs2274700) in the *CFH* gene were assessed by genotyping assay. The information regarding systemic conditions and lifestyle including smoking were documented in each subject by standardized questionnaire.

Results. The intronic SNP (rs1410996) and the synonymous SNP (rs2274700) were associated with a significant risk of AMD ($P = 2.37 \times 10^{-5}$ and 3.52×10^{-5}, respectively). A significant association was also noted between a coding variant (rs800292, I62V) and AMD ($P = 8.63 \times 10^{-6}$). In contrast, the Y402H variant showed no significant association with AMD ($P = 0.101$). Two common haplotypes also demonstrated significant association with AMD ($P = 1.08 \times 10^{-3}$ and 2.00×10^{-5}). Among the environmental factors, smoking alone had a significant association with AMD ($P = 1.17 \times 10^{-4}$).

Conclusions. Although the Y402H variant was not significantly associated with AMD, other coding and noncoding variants in the *CFH* gene including rs1410996 and smoking moderately influenced the risk of AMD in a Japanese population. (*Invest Ophthalmol Vis Sci.* 2007;48:5315–5319) DOI:10.1167/iovs.07-0426

From the [1]Department of Ophthalmology, the [3]Division of Endocrinology and Diabetes, Department of Medicine, and the [4]Division of Radio Isotope Laboratory, Biomedical Research Center, Saitama Medical University, Saitama, Japan; and the [2]Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Supported in part by an 8th Japanese Retinitis Pigmentosa Society Grant (KM); an Institutional Grant from the Medical Research Center, Saitama Medical University (TA); and the generous gift of Mrs. Hisae Iwata and Mr. Seiji Iwata.

Submitted for publication April 10, 2007; revised July 3, 2007; accepted September 13, 2007.

Disclosure: **K. Mori**, None; **P.L. Gehlbach**, None; **S. Kabasawa**, None; **I. Kawasaki**, None; **M. Oosaki**, None; **H. Iizuka**, None; **S. Katayama**, None; **T. Awata**, None; **S. Yoneya**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Keisuke Mori, Department of Ophthalmology, Saitama Medical School, 38 Morohongo, Moroyama, Iruma, Saitama 350-0495, Japan; keisuke@saitama-med.ac.jp.

Age-related macular degeneration (AMD) is a prevalent, late-onset disease, estimated to affect more than 50 million people worldwide.[1] It is a complex disease with multiple genetic and environmental risk factors.[2] The most consistently replicated environmental risk factor is cigarette smoking, and the complement factor H (*CFH*) polymorphism is currently the most consistently replicated genetic risk factor.[2–9] Very recent reports demonstrate that the second variants in locus *LOC387715/HTRA1* also convey susceptibility to AMD, especially the wet form of disease.[10–14]

The association of the *CFH* Y402H variant with AMD has been replicated independently in diverse ethnic groups worldwide—for example, in French, English, Italian, Finnish, and Indian populations.[15–19] However, several Japanese case–control studies have not achieved significance when examining for association between the Y402H variant and AMD.[20–23] Although there is a report demonstrating the association of the Y402H variant with AMD in a Taiwan Chinese population,[24] it remains controversial, as a separate report evaluating a Hong Kong Chinese population indicates that this group more closely resembles *CFH* variants in a Japanese population than in a Western Caucasian population.[25] These persisting uncertainties in Asian populations may result in part from a low incidence of minor allele frequency (MAF) of the Y402H variant in these Asian populations. Grassi et al.[26] have reported the MAF (the risk C allele frequency) in normal populations of different ethnicity, and they are: Japanese 0.07 ± 0.04, Hispanics 0.17 ± 0.03, African-Americans 0.35 ± 0.04, Caucasians 0.34 ± 0.03, and Somalis 0.34 ± 0.03. This result is consistent with the international human haplotype map (HapMap) project database.[27] The phenotypic and epidemiologic characteristics of Asian AMD have been reported and include male predominance, unilateral presentation, a comparatively low incidence of soft drusen, and a greater prevalence of wet AMD.[28–31] It has been proposed that the weaker association of *CFH* gene variants with Japanese AMD is consistent with the ethnic variation in phenotype and epidemiology.[21] Therefore, it is important to examine other polymorphisms present in the *CFH* gene to more fully understand the role of CFH in the molecular pathogenesis of AMD in Asian populations.

Recently, two studies have independently reported *CFH* variations other than Y402H that are strong influences on the risk of AMD.[32,33] It has also been reported that the intronic single nucleotide polymorphisms (SNPs) of *CFH* were more associated with AMD than the Y402H variant.[7,8] Composite likelihood analysis using a high-resolution linkage disequilibrium (LD) map also indicated that there is at least one, and most likely several, variations other than Y402H, that determine the manifestation of disease in AMD.[34]

The purpose of this study was to investigate whether four coding and noncoding variants of the *CFH* gene, including rs1410996, are associated with AMD in native, unrelated Japanese patients. In addition, we examined environmental risk factors associated with Japanese AMD and describe possible gene–environment interactions.

TABLE 1. Allele and Genotype Distribution, Association, and Odds Ratio for the SNPs in the *CFH* Gene

dbSNP ID (Designation)			Case	Control	*P*	OR (95% CI)*
rs800292 (Exon 2; 162V)	Allele	G	275 (73.1)	157 (56.5)	8.63×10^{-6}	0.48 (0.34–0.66)
		A	101 (26.9)	121 (43.5)		
	Genotype	GG	102 (54.3)	42 (30.2)	5.20×10^{-4}†	0.51 (0.35–0.75)
		GA	71 (37.8)	73 (52.5)		
		AA	15 (8.0)	24 (17.3)		
rs1061170 (Exon 9; Y402H)	Allele	T	343 (91.2)	263 (94.6)	0.101	0.59 (0.32–1.11)
		C	33 (8.8)	15 (5.3)		
	Genotype	TT	158 (84.0)	124 (89.2)	0.246†	0.66 (0.33–1.34)
		CT	27 (14.4)	15 (10.8)		
		CC	3 (1.6)	0 (0)		
rs1410996 (Intron 14)	Allele	C	251 (66.8)	140 (50.4)	2.37×10^{-5}	0.51 (0.37–0.69)
		T	125 (33.2)	138 (49.6)		
	Genotype	CC	83 (44.1)	33 (23.7)	3.13×10^{-4}†	0.51 (0.35–0.73)
		CT	85 (45.2)	74 (53.2)		
		TT	20 (10.6)	32 (23.0)		
rs2274700 (Exon 10; synonymous)	Allele	C	251 (66.8)	141 (50.7)	3.52×10^{-5}	0.51 (0.37–0.70)
		T	125 (33.2)	137 (49.3)		
	Genotype	CC	83 (44.1)	33 (23.7)	4.23×10^{-4}†	0.51 (0.35–0.74)
		CT	85 (45.2)	75 (54.0)		
		TT	20 (10.6)	31 (22.3)		

Data are expressed as the number of subjects (% of the entire group).
* Odds ratio (95% confidence interval).
† Age-, gender-adjusted probabilities, and odds ratios by logistic regression analysis.

METHODS

Subjects

The 327 case–control samples were composed of 188 consecutive cases with AMD ranging in age from 51 to 87 years (71.4 ± 8.3; mean ± SD), and 139 control subjects without AMD ranging in age from 52 to 88 years (67.1 ± 11.1), recruited from outpatients visiting the Department of Ophthalmology, Saitama Medical University Hospital, Saitama prefecture, Japan. All case–control subjects were unrelated Japanese Asian. The study was approved by the Ethics Committee of Saitama Medical University, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Each individual was fully informed of the purpose of and the procedures involved in the study. Informed written consent was obtained from each patient.

Ophthalmic Examination and Definition and Classification of AMD

All patients with AMD and the control subjects underwent a full ophthalmic examination, including slitlamp biomicroscopy, funduscopy, and contact lens biomicroscopic examinations of the retina. All patients with AMD had fluorescein and/or indocyanine green fundus angiography. Information regarding diet, family history, systemic conditions and lifestyle, including smoking, was documented in each subject. Inclusion criteria were as follows: age 50 years or older; diagnosis of AMD in one or both eyes; and no association with other retinochoroidal diseases such as angioid streaks, high myopia (greater than 6 D of myopic refractive error), central serous chorioretinopathy, or presumed ocular histoplasmosis. AMD subtypes were diagnosed and classified using the AREDS criteria (groups 1 to 5).[2] In greater detail, neovascular AMD (group 5) was defined by ophthalmoscopic and angiographic findings of classic or occult choroidal neovascularization, serous or hemorrhagic RPE detachments, sensory serous or hemorrhagic retinal detachment, or fibrovascular disciform scarring. Nonneovascular AMD (group 3) includes one or more large drusen or extensive intermediate drusen in at least one eye. There were 168 patients with neovascular AMD (group 5) and 20 patients with nonneovascular AMD (group 3), but no patient with geographic atrophy (group 4). The control subjects (group 1 or 2) were confirmed not to have clinical evidence of AMD by the same complete ophthalmic examination and the same criteria used to identify the study cohort of patients with AMD.

Genotyping and Statistical Analysis

Genomic DNA was extracted from the peripheral blood of each individual using a DNA extraction and purification kit (Wizard Genomic DNA Purification Kit; Promega, Madison, WI), according to the manufacturer's instructions. The samples were genotyped (*Taq*Man genotyping assay with the ABI Prism 7000 Sequence Detection System; Applied Biosystems, Inc. [ABI], Foster City, CA) and the data were analyzed (Allelic Discrimination Program; ABI).

TABLE 2. Linkage Disequilibrium (LD) Map

	rs800292	rs1061170	rs1410996	rs2274700
rs800292		0.0292	0.644	0.649
rs1061170	6.01×10^{-06}		0.0533	0.0529
rs1410996	2.14×10^{-93}	3.57×10^{-09}		0.994
rs2274700	5.05×10^{-94}	4.00×10^{-09}	2.41×10^{-143}	

Values forming upper-right triangle: r^2; values forming lower-left triangle: *P* values. Dark gray shading: strong LD, $r^2 > 0.80$; light gray shading: moderate LD, $r^2 > 0.50$.

TABLE 3. Haplotype Frequencies and Association for the SNPs in *CFH* Gene

Haplotypes	Case	Control	χ^2	P*	P_{corr}†
H1: G-T-C-C	0.566	0.437	10.62	1.12×10^{-3}	1.08×10^{-3}
H2: A-T-T-T	0.254	0.416	19.05	1.28×10^{-5}	2.00×10^{-5}
H3: G-T-T-T	0.078	0.077	2.69×10^{-3}	0.959	0.970
H4: G-C-C-C	0.087	0.047	3.99	0.046	0.055

H1, H2, H3, and H4 demonstrate four haplotypes with a frequency >0.03, defined by four SNPs; rs800292, rs1061170, rs1410996, and rs2274700, respectively.

* Chi-square test.

† Corrected *P* by permutation test (number of iterations, 1,000,000).

Genotype and allele frequencies between cases and controls were compared by using the χ^2 test for quality of proportions. Hardy-Weinberg equilibrium tests were performed by χ^2 analysis. Logistic regression analysis was used to estimate the odds ratio (ORs) and corresponding 95% confidence interval (95% CI) for the effect of genotypes and environmental risk factors on the risk of AMD development, by adjusting covariate effects for age and gender. The interaction of cigarette smoking (pack-year) and the tested SNPs was evaluated by using a logistic regression analysis and by adjusting covariate effects for age and gender—that is, the SNPs (major homozygous, 2; heterozygous, 1; and minor homozygous, 0; multiplicative model), the pack-year and the product of them were included as explanatory variables in a logistic regression model, and then the interactive effects were considered to be represented by the increased OR for the product. Other genetic models (dominant: major homozygous or heterozygous, 1, minor homozygous, 0; recessive: major homozygous, 1, heterozygous or minor homozygous, 0) were also tested for each SNP. Inferred haplotype frequencies by the expectation–maximization (EM) algorithm of the SNPs were compared by using permutation tests. PAWE software was used for the power analysis of the case–control study.[35] $P < 0.05$ was considered to be statistically significant. All analysis was performed with commercially available software (SNPAlyze ver. 6.0.1; Dynacom, Chiba, Japan; and SSRI ver. 1.20, SSRI, Tokyo, Japan).

RESULTS

The allele and genotype distribution, association, and odds ratio of rs800292, rs1061170, rs1410996, and rs2274700 are given in Table 1. The genotype frequencies for cases and controls of all tested SNPs were in Hardy-Weinberg equilibrium ($P > 0.3$). A significant association was noted between a nonsynonymous variant (rs800292, I62V) and AMD ($P = 8.63 \times 10^{-6}$, OR: 0.48, 95% CI: 0.34–0.66). The intronic SNP (rs1410996) and the synonymous SNP (rs2274700) were also associated significantly with development of AMD ($P = 2.37 \times 10^{-5}$, OR: 0.51, 95% CI: 0.37–0.69; $P = 3.52 \times 10^{-5}$, OR: 0.51, 95% CI: 0.37–0.70, respectively). In contrast, the Y402H (rs1061170) variant showed no significant association with AMD ($P = 0.101$). However, the power (allelic test) of this study was estimated to be 40% at a maximum for rs1061170 (Y402H) to detect a statistically significant difference ($P = 0.05$) by the power analysis. Probabilities and ORs of rs800292, rs1061170, rs1410996, and rs2274700, adjusted for age and gender using logistic regression analysis were $P = 5.20 \times 10^{-4}$, OR: 0.51, 95% CI: 0.35–0.75; $P = 0.246$, OR: 0.66, 95% CI: 0.33–1.34, $P = 3.13 \times 10^{-4}$, OR: 0.51, 95% CI: 0.35–0.73; $P = 4.23 \times 10^{-4}$, OR: 0.51, 95% CI: 0.35–0.74, respectively (Table 1).

The significantly associated SNPs showed high LD. Two SNPs (rs1410996 and rs2274700) fell into virtually complete LD ($r^2 = 0.994$, $P = 2.41 \times 10^{-143}$). The I62V variant (rs800292) is also in moderately high LD with rs1410996 and rs2274700. However, The Y402H variant (rs1061170) showed relatively low LD with other SNPs (Table 2). Two haplotypes, GTCC and ATTT demonstrated significant association with AMD (the corrected probabilities by permutation test = 1.08×10^{-3}, 2.00×10^{-5}, respectively). One haplotype, GTCC, was associated with disease susceptibility and the other, ATTT, appeared to be protective (Table 3). The results were consistent with individual SNP analysis.

Among several environmental factors cigarette smoking showed a significant association with development of AMD using logistic regression analysis by adjusting covariate effects for other factors including age, gender, cigarette smoking, body mass index, heart disease, and hypertension ($P = 1.17 \times 10^{-4}$, OR: 2.03, 95% CI: 1.41–2.90). The incidence of AMD was slightly but not significantly associated with body mass index (BMI; Table 4). Gene–environment interactions of cigarette smoking (pack-year) with tested SNPs in a multiplicative model were assessed by logistic regression analysis. No significant interaction was found between cigarette smoking and each tested SNP in the multiplicative model: rs800292, rs1061170, rs1410996, and rs2274700 ($P = 0.177$, 0.081, 0.196, and 0.176, respectively). There was also no significant interaction when tested by other genetic models (Table 5).

TABLE 4. Environmental Factors Association with AMD

	Case	Control	P	OR (95% CI)*
Age (y)	71.4 ± 8.3	67.1 ± 11.1	2.29×10^{-3}	1.05 (1.02–1.08)
Gender (% men)†	76.1	52.1	0.480	0.79 (0.41–1.52)
Smoking (pack-year)	26.4 ± 27.0	12.1 ± 19.1	1.17×10^{-4}	2.03 (1.41–2.90)
Body mass index (BMI, kg/m^2)	23.2 ± 3.1	22.6 ± 3.3	0.084	1.08 (0.99–1.17)
Heart diseases (%)‡	23.9	18.5	0.904	1.04 (0.55–1.96)
Hypertension (%)§	42.8	30.3	0.409	1.26 (0.73–2.17)

Data in the Case and Control columns are expressed as the mean ± SD or as a percentage of the group.

* Multivariate (age, gender, smoking, BMI, heart diseases, hypertension)-adjusted odds ratio (95% CI).

† Male, 0; female, 1.

‡ None, 0; present, 1.

§ None, 0; present, 1.

Table 5. Gene-Environment Interaction between Tested SNPs and Smoking History

dbSNP ID	*P*	OR (95% CI)
rs800292	0.177	0.988 (0.970–1.006)
rs1061170	0.081	1.024 (0.997–1.051)
rs1410996	0.196	0.987 (0.969–1.006)
rs2274700	0.176	1.013 (0.994–1.032)

The interaction of the tested SNPs and cigarette smoking (pack-year) was evaluated by logistic regression, adjusting covariate effects for age and gender.

Discussion

This study was sufficiently powered to demonstrate a clear association between AMD and several variants in the *CFH* gene, including rs800292 (I62V), rs1410996, and rs2274700 in a Japanese population. We did not demonstrate a statistically significant association between the rs1061170 (Y402H) variant and AMD. The results were not substantially different after evaluation with the more conservative Bonferroni correction. The significantly associated SNPs showed moderate to strong LD and two SNPs (rs1410996 and rs2274700) fell into virtually complete LD. Two haplotypes, one associated with disease susceptibility and one that seemed to be protective, demonstrated significant association with AMD. However, the power (allelic test) of this study was estimated to be 40% at a maximum for rs1061170 (Y402H) to detect a statistically significant difference ($P = 0.05$). Although the contribution of the Y402H *CFH* polymorphism to Japanese AMD remains a point for further large-scale studies, the findings reported indicate that other *CFH* gene variants may play a comparatively larger role in development of AMD in the Japanese population.

Ethnic variation in the prevalence and phenotypic spectrum of AMD has been well described in studies of diverse populations.[28–31,36–38] For example, in the Japanese, serous pigment epithelial detachment is a stronger indicator of AMD than is soft drusen. This finding is in part supported by the relative prevalence of wet AMD in the fellow eye being 58% and 18%, respectively, for each of these indicators.[39] One hypothesis for this ethnic variation is that there is a relatively small contribution from the *CFH* gene in Japanese AMD and that this differential contribution may correlate with the phenotypic and epidemiologic differences seen.[21] The results of allele frequencies and their associations (probabilities) with AMD among Japanese, Chinese, and Caucasians are summarized in Table 6. The risk allele frequencies of I62V- and Y402H-coding variants are more similar between Japanese and Chinese populations. This is consistent with the previous reports.[21,23,25] Our data provide additional information that two SNPs (rs1410996 and rs2274700) in complete LD show significant association with AMD. The C allele frequency of these SNPs (rs1410996 and rs2274700) are 0.668 and 0.668 in cases and 0.504 and 0.507 in controls, respectively, which is slightly less frequent than those in Caucasian cases and controls.[32] The context of rs1410996 is intron 14 and rs2274700 is exon 10, but synonymous. Currently, the molecular role of these SNPs in AMD is unclear. One hypothesis is that these or other SNPs in the LD group modulate AMD risk by regulating the expression of *CFH* , of other nearby complement genes, or both, rather than by disrupting the function of the CFH protein.[32]

To further understand the pathogenesis of AMD development in the Japanese population we have evaluated the risk associated with several potential environmental risk factors for AMD. Among the tested factors, cigarette smoking showed a significant association with development of AMD. This result is consistent with several population-based studies worldwide that have included a Japanese population.[4,37] There are several controversies regarding gene–environment interactions of AMD. Rivera et al.[11] demonstrated no significant differences in risk allele frequencies for *CFH* between smokers and nonsmokers, whereas Despriet et al.[40] showed the combined effect of homozygosity for the *CFH* Y402H variant and cigarette smoking exceeds the sum of the independent effects. Two other groups have also found no statistically significant interaction between the *CFH* genotype and cigarette smoking.[41,42] Although several questions remain to be answered about gene–environment interactions pertaining to *CFH* variants and cigarette smoking, our results provide no significant interaction between cigarette smoking and each tested of the SNPs: rs800292, rs1061170, rs1410996, and rs2274700. However, there is no doubt that further study is needed to confirm these gene–environment interaction results.

In summary, although we failed to demonstrate a significant association of the Y402H variant with AMD, other coding and noncoding variants in the *CFH* gene including rs1410996 moderately influenced the risk of AMD in the Japanese population studied. Not surprisingly, our methods also revealed that smoking was a risk factor for AMD in this population. Taken collectively, our data provide further support for ethnic variation in the risk of AMD associated with the *CFH* gene. We provide evidence that the phenotypic and epidemiologic variation present is in part attributable to differential effects of various polymorphisms in the *CFH* gene. We have not commented on the molecular biological or physical basis of this interaction, but propose area of study as one of several logical next avenues of investigation that will lead to a better understanding of ethnic differences in the manifestation of common disease.

Table 6. CFH Association Comparisons among Japanese, Chinese, and Caucasians

		Japanese			Chinese	Caucasian	
		Gotoh[21]	Fuse[23]	This Study	Chen[25]	Li[32]	Maller[33]
N (case:control)		251 (146:105)	272 (80:192)	327 (188:139)	407 (163:244)	994 (726:268)	2172 (1238:934)
rs800292	Case	0.71 (0.013)	0.694 (0.62)	0.735 (8.63×10^{-6})	0.752 (8×10^{-5})	0.902 ($<1 \times 10^{-13}$)	NA (2.02×10^{-20})
	Control	0.60	0.667	0.565	0.619	0.762	NA
rs1061170	Case	0.08 (0.074)	0.044 (0.25)	0.088 (0.10)	0.058 (0.20)	0.616 ($<1 \times 10^{-25}$)	0.615 (1.79×10^{-59})
	Control	0.04	0.073	0.053	0.039	0.340	0.359
rs1410996	Case			0.668 (2.37×10^{-5})		0.843 ($<1 \times 10^{-29}$)	0.808 (2.65×10^{-61})
	Control			0.504		0.538	0.571
rs2274700	Case	0.65 (0.015)		0.668 (3.52×10^{-5})		0.842 ($<1 \times 10^{-30}$)	
	Control	0.54		0.507		0.554	

Data are expressed as the risk allele frequencies (*P*).

References

1. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol*. 2004;137:486–495.
2. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration: a case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. *Ophthalmology.* 2000;107:2224–2232.
3. Age-Related Eye Disease Study Research Group. Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study. AREDS report no. 19. *Am J Ophthalmol*. 2005;112:533–539.
4. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: pooled findings from three continents. *Ophthalmology*. 2001;108:697–704.
5. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
6. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.
7. Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.
8. Zareparsi S, Branham KEH, Li M, et al. Strong association of the Y402H variant in complement factor H at 1q32 with susceptibility to age-related macular degeneration. *Am J Hum Genet*. 2005;77:149–153.
9. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA*. 2005;102:7227–7232.
10. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet*. 2005;77:389–407.
11. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14:3227–3236.
12. Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am J Hum Genet*. 2006;78:852–864.
13. Dewan A, Liu M, Hartman S, et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science*. 2006;314:989–992.
14. Yang Z, Camp NJ, Sun H, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science*. 2006;314:992–993.
15. Souied EH, Levezeil N, Richard F, et al. Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population. *Mol Vis*. 2005;11:1135–1140.
16. Sepp T, Khan JC, Thurlby DA, et al. Complement factor H variant Y402H is a major risk determinant for geographic atrophy and choroidal neovascularization in smokers and nonsmokers. *Invest Ophthalmol Vis Sci*. 2006;47:536–540.
17. Simonelli F, Frisso G, Testa F, et al. Polymorphism p. 402Y>H in the complement factor H protein is a risk factor for age related macular degeneration in an Italian population. *Br J Ophthalmol*. 2006;90:1142–1145.
18. Seitsonen S, Lemmela S, Holopainen J, et al. Analysis of variants in the complement factor H, the elongation of very long chain fatty acids-like 4 and the hemicentin 1 genes of age-related macular degeneration in the Finnish population. *Mol Vis*. 2006;12:796–801.
19. Kaur I, Hussain A, Hussain N, et al. Analysis of CFH, TLR4, and APOE polymorphism in India suggests the Tyr402His variant of CFH to be a global marker for age-related macular degeneration *Invest Ophthalmol Vis Sci*. 2006;47:3729–3735.
20. Okamoto H, Umeda S, Obazawa M, et al. Complement factor H polymorphisms in Japanese population with age-related macular degeneration. *Mol Vis*. 2006;12:156–158.
21. Gotoh N, Yamada R, Hiratani H, et al. No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese. *Hum Genet*. 2006;120:139–143.
22. Uka J, Tamura H, Kobayashi T, et al. No association of complement factor H gene polymorphism and age-related macular degeneration in the Japanese population. *Retina*. 2006;26:985–987.
23. Fuse N, Miyazawa A, Mengkegale M, et al. Polymorphisms in complement factor H and hemicentin-1 genes in a Japanese population with dry-type age-related macular degeneration. *Am J Ophthalmol*. 2006;142:1074–1076.
24. Lau LI, Chen SJ, Cheng CY, et al. Association of the Y402H polymorphism in complement factor H gene and neovascular age-related macular degeneration in Chinese patients. *Invest Ophthalmol Vis Sci*. 2006;47:3242–3246.
25. Chen LJ, Liu DT, Chan WM, et al. Association of complement factor H polymorphisms with exudative age-related macular degeneration. *Mol Vis*. 2006;12:1536–1542.
26. Grassi MA, Fingert JH, Scheetz TE, et al. Ethnic variation in AMD-associated complement factor H polymorphism p.Tyr402His. *Hum Mutat*. 2006;27:921–925.
27. Consortium TIH. The International HapMap Project. *Nature*. 2003;426:789–796.
28. Chang TS, Hay D, Courtright P. Age-related macular degeneration in Chinese-Canadians. *Can J Ophthalmol*. 1999;34:266–271.
29. Uyama M, Wada M, Nagai Y, et al. Polypoidal choroidal vasculopathy: natural history. *Am J Ophthalmol*. 2002;133:639–648.
30. Sho K, Takahashi K, Yamada H, et al. Polypoidal vasculopathy: incidence, demographic features, and clinical characteristics. *Arch Ophthalmol*. 2003;121:1392–1396.
31. Bird AC. The Bowman lecture: towards an understanding of age-related macular disease. *Eye*. 2003;17:457–466.
32. Li M, Atmaca-Sonmez P, Othman M, et al. CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. *Nat Genet*. 2006;38:1049–1054.
33. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nat Genet*. 2006;38:1055–1059.
34. Ennis S, Goverdhan S, Cree A, Hoh J, Collins A, Lotery AJ. Fine-scale linkage disequilibrium mapping of age related macular degeneration in the complement factor H gene region. *Br J Ophthalmol.* 2007;91:966–970.
35. Gordon D, Finch SJ, Nothnagel M, Ott J. Power and sample size calculations for case-control genetic association tests when errors are present: application to single nucleotide polymorphisms. *Hum Hered*. 2002;54:22–33.
36. Hsu WM, Cheng CY, Liu JH, Tsai SY, Chou P. Prevalence and causes of visual impairment in an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology*. 2004;111:62–69.
37. Miyazaki M, Kiyohara Y, Yoshida A, Iida M, Nose Y, Ishibashi T. The 5-year incidence and risk factors for age-related maculopathy in a general Japanese population: the Hisayama study. *Invest Ophthalmol Vis Sci*. 2005;46:1907–1910.
38. Schachat AP, Hyman L, Keske MC, Connell AM, Wu SY. Features of age-related macular degeneration in a black population: the Barbados Eye Study Group. *Arch Ophthalmol*. 1995;113:728–735.
39. Uyama M, Takahashi K, Ida N, et al. The second eye of Japanese patients with unilateral exudative age related macular degeneration. *Br J Ophthalmol*. 2000;84:1018–1023.
40. Despriet DD, Klaver CC, Witteman JC, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA.* 2006;296:301–309.
41. Conley YP, Jakobsdottir JH, Mah T, et al. CFH, ELOVL4, PLEKHA1 and LOC387715 genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses. *Hum Mol Genet*. 2006;15:3206–3218.
42. Seddon JM, George S, Rosner B, Klein ML. CFH gene variant, Y402H, and smoking, body mass index, environmental associations with advanced age-related macular degeneration. *Hum Hered*. 2006;61:157–165.