Molecular Vision 2006; 12:1536-42 <http://www.molvis.org/molvis/v12/a176/>
Received 8 July 2006 | Accepted 27 November 2006 | Published 5 December 2006

Association of complement factor H polymorphisms with exudative age-related macular degeneration

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Purpose: Variants in the complement factor H (CFH) gene have been reported to be associated with age-related macular degeneration (AMD). We conducted a case-control association study to investigate the association of 6 single nucleotide polymorphisms (SNPs) in CFH with exudative AMD in the Chinese population.
Methods: We recruited 163 cases and 244 controls, all ethnic Chinese, with complete ophthalmic examination including fundus investigation. Cigarette smoking was recorded. Six SNPs (dbSNP ID: rs3753394, rs800292, rs1061147, rs1061170, rs380390, and rs1329428) in the CFH gene were genotyped by Taqman assays.
Results: Y402H (1277 T>C) has low frequencies in our study population, 5.8% in patients and 3.9% in controls. It was not associated with exudative AMD adjusted for age, gender and smoking. Significant associations were detected for AMD with rs3753394 (p=0.003, p_{corr}=0.018), rs800292 (p=0.00053, p_{corr}=0.0032), and rs1329428 (p=0.00092, p_{corr}=0.0028), p_{corr} values obtained after adjustment for multicomparison. A haplotype containing these four SNPs (TGTC) was found to confer a significantly increased likelihood of exudative AMD with an odds ratio of 1.68 (95% CI: 1.26-2.23) p=0.0003 (p_{corr}=0.0026 after correction by permutation test). Logistic regression analysis detected no interactions between the SNPs and age, gender or smoking.
Conclusions: We have found differences in the association between the CFH gene and exudative AMD in Chinese from Caucasians and Japanese. We detected SNP rs3753394 in the CFH promoter carrying a significantly increased risk for exudative AMD.

Age-related macular degeneration (AMD) is a late-onset, chronic and progressive degenerative disorder in the macular at the central region of the retina. A common eye disease estimated to affect about 50 million people worldwide [1], it is also the leading cause of visual impairment and blindness in people aged more than 65 years in developed countries [2,3]. Among those patients with severe visual impairment, nearly 90% are caused by exudative AMD, which is the so-called wet form and a subtype of late-stage AMD found in approximately 10% of all AMD cases. Exudative AMD is characterized by choroidal neovascularization beneath the retinal pigmented epithelium (RPE) or between the RPE and the retina [4,5]. In the Chinese population of Hong Kong, AMD was observed in 5.9% of subjects aged 40 years or above [6]. In the Chinese population in Beijing, AMD contributed to 2.0% of low vision and 7.7% blindness of people more than 40 years old [7]. A study in Taiwan Chinese on 48 participants with visual impairment aged between 65 and 91, found 5 (10.4%) of them of low vision or blind due to AMD [8]. Although right now AMD is not the leading cause of visual impairment in China [6-8], its effects are growing as the population ages.

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The etiology of AMD is complex and multifactorial, likely resulting from interaction between environmental factors and multigenetic factors [3]. The inflammatory pathway or the immune system may play a role [5]. Late disease onset, rarity of big family pedigrees and clinical heterogeneity have rendered cloning of AMD genes difficult. Research attempts using candidate gene screening, positional cloning, and full genome scan have defined association of some allelic variants with AMD, including complement factor H (*CFH*) [9-17], *LOC387715* [18], *ABCA4* [19], *APOE* [20], *FBLN5* [21], *ELOVL4* [22], and *TLR4* [23].

The *CFH* gene has recently been shown to be strongly associated with AMD [9-17]. The missense variant, Y402H, increases a significant risk for AMD in Caucasian populations. However, it was not associated with AMD in Japanese [24,25]. Other variants in the *CFH* gene, including the I62V, were also reported to be associated with AMD in both Caucasians and Japanese [12,24]. Variants L9H, R32Q in the factor B (*FB*) gene and E318D in the complement component 2 (*C2*) gene have been shown to confer a significantly reduced risk of AMD [26]. All these findings suggested a role of the complement system in the molecular pathogenesis of AMD.

We had previously studied the association between the *ABCA4* and *APOE* genes and AMD. We found *ABCA4* splicing variations just associated with a small proportion of Chinese AMD cases and *ABCA4* sequence alterations not linked with AMD [27]. Our genotype results also showed that ApoE

was unlikely to be a major risk factor of AMD [28]. In this present study we investigated the association between *CFH* and exudative AMD by genotyping six of representative single nucleotide polymorphisms (SNPs). Five of them (dbSNP ID: rs800292, rs1061147, rs1061170, rs380390, and rs1329428) have been reported to be associated with AMD [9,12]. rs3753394 was selected because it is the only SNP reported in the *CFH* promoter, though not associated with AMD in Caucasians [11,12]. Possible interactions between the SNPs and possible environment risk factors were also studied by logistic regression.

METHODS

Study population: All study subjects were recruited at the Eye Clinic of the Prince of Wales Hospital, Hong Kong. They all gave informed consent. The study protocol was approved by the Ethics Committee on Human Research, the Chinese University of Hong Kong. All the procedures used conformed to the tenets of the Declaration of Helsinki. All participants received a standard examination protocol and visual-acuity measurement. Slitlamp biomicroscopy of the fundi was performed by an experienced ophthalmologist. Stereoscopic color fundus photographs were taken by a trained ophthalmic photographer. Grading was based on the standard classification described by the International Age-related Maculopathy Epidemiological Study Group [29]. Patients with exudative AMD had nondrusenoid RPED (retinal pigment epithelium detachment), CNV (choroidal neovascularization), serous or hemorrhagic retinal detachments, subretinal or sub-RPE hemorrhage or fibrosis. In total, 163 exudative AMD patients were recruited, including 88 males and 75 females. The age at diagnosis ranged from 60-94 years, mean 75.5±7.5 years. Control subjects were recruited from patients with no sign of AMD or any other eye diseases except mild senile cataracts. Subjects with severe cataracts were excluded from recruitment. A total of 244 control subjects with 114 males and 130 females were included in this study, age ranging from 60-99 years, mean 73.5±6.5 years. The cases and controls were gender matched. Smoking status was inquired and recorded during history taking. A smoker was defined as a person who smoked at least 5 cigarettes daily for more than one year [30]. Data of smoking status was available from 153 cases and 190 controls. They were classified as never-smokers, experienced smokers and current smokers.

Genotyping: Peripheral venous blood was collected from all subjects for genomic DNA extraction using a QIAamp DNA Blood kit (Qiagen, Valencia, CA). The SNPs were genotyped using TaqMan genotyping assays on an ABI Prism 7000 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA).

Statistical analysis: Hardy-Weinberg equilibrium (HWE) for each polymorphism was tested by χ^2 test. Allele or genotype frequencies between cases and controls were compared by χ^2 analysis. The unadjusted odds ratios of the alleles and genotypes were estimated by the χ^2 test. Analysis of variance (ANOVA) was used to compare the age at diagnosis of the patients carrying different genotypes. Statistical significance was defined as $p<0.05$. P values were corrected by the Bonferroni test for multiple comparisons.

Logistic regression analysis was applied to study the association of *CFH* polymorphisms with AMD risk factors of age, gender and smoking. All genotypes were set as categorical variables (homozygote with major allele: 2, heterozygote: 1, homozygote with minor allele: 0) as well as the age (60-69: 0, 70-79: 1, greater than or equal to 80: 2), gender (male: 0, female: 1), and smoking status (non-smoking: 0, experienced smoking: 1, current smoking: 2). Multiple logistic regression models were built and optimized using a "backward stepwise" approach. Goodness of fit of the model was assessed by the Hosmer and Lemeshow test [31]. Odds ratios (OR) of each risk factor and the corresponding 95% confidence interval (CI), adjusted for other risk factors, were calculated with the logistic regression method. Logistic regression models were also built using various *CFH* SNPs to search for the possible interaction between the CFH SNPs and possible risk factors of age, gender and smoking. A stepwise regression approach was used to optimize the analysis. The SPSS statistical software was used (SPSS ver.11.0; SPSS Inc., Chicago, IL).

Pairwise linkage disequilibrium (LD, D') estimation between polymorphisms and EM-based haplotype association analysis were performed using Haploview (ver 3.31).

RESULTS

Single nucleotide polymorphysm analysis: Unequivocal results were obtained in all study samples for the SNP genotyping. The genotype frequencies of the controls followed Hardy-Weinberg equilibrium. Between exudative AMD (n=163) cases and controls (n=244), significant association was detected at rs3753394, rs800292 and rs1329428 but not at rs1061147, rs1061170 or rs380390 after adjustment for multi-comparisons (Table 1). The latter three SNPs were in complete linkage disequilibrium, among them the rs1061170 (Y402H) was the only SNP that leads to a non-synonymous amino acid change. The frequency for the rare allele C at Y402H was 4.67% in our study population (5.8% in exudative AMD cases and 3.9% in controls, with no significant difference). The major allele of three other SNPs (T for rs3753394, G for rs800292, and C for rs1329428) conferred a 1.6 (95% CI: 1.2-2.2) fold, 1.9 (95% CI: 1.4-2.5) fold and 1.8 (95% CI: 1.4-2.5) fold of increased likelihood of exudative AMD, respectively. Individuals with two copies of the risk alleles carried a significantly increased likelihood of disease compared with individuals with only one or no copy of the risk allele. Individuals with one copy of the risk allele at rs1329428 carried a significantly increased likelihood of disease compared with individuals with no copy. However, individuals with one copy of the risk allele at SNP rs3753394 and rs800292 carried a slightly but insignificantly increased likelihood of disease (Table 1).

To investigate whether there are other AMD associated polymorphisms in the *CFH* promoter, we randomly selected 48 cases with exudative AMD and 46 controls for direct sequencing after PCR with primers: P1F (AGA ATC GTG GTC TCT GTG TGT GG) and P1R (AGC AGC TGG TGA TAT

CCT CTG G) for sequence from -794 to -248. P2F (TCAAATGAGAGTGAGCCAGTTGC) and P2R (CTG TTC ACA ACG TCC AGT TCT CC) for sequence -541 to +36. No sequence alterations except rs3753394 were detected.

The mean age of the patients carrying a homozygous risk genotype were 1.2-3.2 years younger than those who carried a homozygous variant genotype [75.6±8.0 (TT) versus 77.7±6.8 (CC) at rs3753394, 75.2±7.8 (GG) versus 78.4±7.3 (AA) at rs800292 and 75.2±7.5 (CC) versus 76.4±6.6 (TT) at rs1329428]. However, the differences were not statistically significant by ANOVA. Mean age at diagnosis was almost identical in cases with heterozygous genotypes and homozygous risk genotypes.

Linkage disequilibrium and haplotype association analysis: Pairwise LD analysis showed rs800292 in high LD with rs3753394 (D'=0.903, confidence 0.85-0.94) and rs1329428 (D'=0.872, confidence 0.82-0.91). But it is in low LD with rs1061147, rs1061170 and rs380390 (D'=0.38, confidence 0.04-0.77). Two haplotypes, TGTC and CATT, were significantly associated with exudative AMD. Haplotype TGTC, containing all the major alleles of rs3753394, rs800292, rs1061170 and rs1329428, conferred a 1.68 (95% CI: 1.26-2.23) fold increased likelihood of exudative AMD. The results were consistent with individual SNPs analysis (Table 1). Comparison of *CFH* haplotypes among Caucasians, Japanese and Chinese revealed ethnic differences (Table 2).

Logistic regression analysis: Results of backward stepwise logistic regression analysis showed that a model containing age, smoking status, rs3753394 and rs1061170 were of the best goodness of fit (Table 3). The P value from Hosmer and Lemeshow Test was 0.445, indicating that the model prediction did not significantly differ from the observed. In this model, age of greater than or equal to 80 years, cigarette smoking (experienced smoking and current smoking) and genotype TT at SNP rs3753394 were significantly associated with exudative AMD adjusted for other factors. The rs1061170 genotype was still not associated with exudative AMD after adjustment for other factors, but it contributed to optimize the model. Gender, rs800292 and rs1329428 were not included in the model. Since rs3753394, rs800292 and rs1329428 are in high LD, we included the latter two into the logistic regression model by "enter" approach and found that they were also significantly associated with exudative AMD adjusted for age, gender, smoking and rs1061170 (Table 3). Meanwhile, further logistic regression analysis also showed that none of the six SNPs had significant interaction with age, gender and smoking.

DISCUSSION

We have detected differences in the association between the *CFH* gene and exudative AMD in Chinese from Caucasians as well as Japanese, though it was more similar between Chinese and Japanese (Table 2).

SNP rs1061170 (Y402H) has been identified as the major genetic factor for developing AMD in Caucasians. Frequencies of the C allele were between 61-94% in AMD and 34-46% in controls [9-17]. However, the Y402H C allele was low in frequency in our Chinese study population (5.8% in cases and 3.9% in controls). It was not associated with exudative AMD. Actually such low frequency does not allow conclusion of genetic susceptibility in Chinese. In Japanese, the C allele was also at a low frequency and not associated with exudative AMD [24,25]. Dramatic differences may exist in the allele frequencies of individual SNPs across populations [32].

Table 1. *CFH* single nucleotide polymorphysm association with exudative age related macular degeneration

db SNP ID	Designiation	Allele	Allele Distribution(%) Cases	Allele Distribution(%) Controls	Allele Association (P value)	Odds Ratio (95% CI)	Genotype	Genotype Distribution(%) Cases	Genotype Distribution(%) Controls	Genotype Association P value (Pcorr)	Odds Ratio (95% CI)
rs3753394	Promoter (-257)	T	224(68.7)	280(57.4)	0.0011	1.63(1.22~2.19)	TT	83(50.9)	83(34.0)	0.0030	2.14(1.18~3.86)*
		C	102(31.3)	208(42.6)			CT	58(35.6)	114(46.7)	(0.018)	1.09(0.60~1.97)+
							CC	22(13.5)	47(19.3)		1.97(1.27~3.05)++
rs800292	Exon 2 (I62V)	G	245(75.2)	302(61.9)	0.00008	1.86(1.37~2.54)	GG	95(58.3)	96(39.3)	0.00053	2.89(1.45~5.77)
		A	81(24.8)	186(38.1)			AG	55(33.7)	110(45.1)	(0.0032)	1.46(0.72~2.97)
							AA	13(8.0)	38(15.6)		1.98(1.29~3.04)
rs1061147	Exon 7 (A307A)	A	19(5.8)	19(3.9)	0.20	NS	AA	1(0.6)	0(0.0)	0.30	NS
		C	307(94.2)	469(96.1)			AC	17(10.4)	19(7.8)		
							CC	145(89.0)	225(92.2)		
rs1061170	Exon 9 (Y402H)	C	19(5.8)	19(3.9)	0.20	NS	CC	1(0.6)	0(0.0)	0.30	NS
		T	307(94.2)	469(96.1)			CT	17(10.4)	19(7.8)		
							TT	145(89.0)	225(92.2)		
rs380390	IVS15	G	19(5.8)	19(3.9)	0.20	NS	GG	1(0.6)	0(0.0)	0.30	NS
		C	307(94.2)	469(96.1)			CG	17(10.4)	19(7.8)		
							CC	145(89.0)	225(92.2)		
rs1329428	IVS15	C	237(72.7)	289(59.2)	0.00008	1.83(1.35~2.48)	CC	88(54.0)	93(38.1)	0.00092	3.24(1.67~6.30)
		T	89(27.3)	199(40.8)			CT	61(37.4)	103(42.2)	(0.0028)	2.03(1.04~3.99)
							TT	14(8.6)	48(19.7)		1.60(1.04~2.46)

At each single nucleotide polymorphysm, the allele or genotype which has a higher frequency in cases was listed above the rest allele or genotypes. Asterisk (*) represents odds ratio compared the likelihood of age-related macular degeneration (AMD) in individuals with two copies of the risk allele versus individuals with no copy of the risk allele. Vertical arrow (+) represents odds ratio compared the likelihood of AMD in individuals with one copy of the risk allele versus individuals with no copy of the risk allele. Christ symbol (++) represents odds ratio compares the likelihood of AMD in individuals with two copies of the risk allele versus individuals with only one copy of the risk allele. The p values <0.05/6=0.0083 (Bonferroni correction) was considered as statistically significant. NS represents non-significant. p_{corr} values were obtained after adjustment for multicomparison.

In the present study, three alleles, T, G, C, respectively for SNPs in the promoter (rs3753394), exon 2 (rs800292) and intron 15 (rs1329428) located in a common haplotype TGTC (with an estimated haplotype frequency of 56%), significantly increased the susceptibility for exudative AMD. SNP rs1329428 showed a dominant effect on the association with AMD while the rs3753394 and rs800292 showed a recessive effect (Table 1). However, after adjustment for age, gender and smoking by logistic regression, all SNPs showed a recessive effect. Only genotypes with homozygous risk alleles remain significantly associated with exudative AMD. The heterozygous genotypes was not associated with AMD but indicated a trend for disease susceptibility (OR>1, p>0.05). For rs1329428, the C allele was a risk allele for AMD in Caucasians [9]. The C allele frequency is 59% in CEU population (Hapmap project), compared to 64.6% in our overall study subjects (72.7% in cases and 59.2% in controls) and conferred a significantly increased risk for exudative AMD (Table 1). The association between AMD and this SNP was similar between Caucasians and Chinese.

In our study, rs3753394 and rs800292, which were in high LD with rs1329428, were significantly associated with exudative AMD adjusted for age, gender and smoking. The I62V variant (rs800292) was associated with AMD in both Caucasians and Japanese [12,24]. However, this SNP was not associated with exudative AMD in another Japanese study population [25]. We compared the haplotypes among Caucasians, Japanese and Chinese and found that haplotype H1 (GT) was more frequent in Japanese AMD cases and H2 (AT) in Japanese controls (Table 2). While these haplotypes were not as-

Table 2. CFH haplotype comparisons among caucasians, Japanese, and Chinese

	rs800292	rs1061170	Caucasians[12]		Japanese[25]		Chinese		Odds Ratio (95% CI)	P	Pcorr
			Cases	Controls	Cases	Controls	Cases	Controls			
H1	G	T	N/A	N/A	0.62	0.57	0.69	0.58	1.64(1.22~2.20)	0.001	0.0064
H2	A	T	0.12	0.21	0.30	0.40	0.25	0.38	0.54(0.39~0.73)	0.00008	0.0005
H3	G	C	0.50	0.29	0.08	0.03	0.058	0.039	NS	0.20	0.53

All haplotypes with a frequency of >1% in our study population are displayed. Frequencies of corresponding haplotypes in Caucasians and Japanese were referred to the reports of Hageman et al. (2005) [12] and Gotoh et al. (2006) [25]. The AMD cases in Hageman et al [12] included geographic and exudative AMD. Cases for Japanese [25] and Chinese were exudative AMD only. H1 in Caucasians was non-significantly associated with AMD between cases and controls, haplotype frequency was not available. p_{corr} represents corrected p value by permutation test (number of iterations equals to 10,000). NS represents non-significant. N/A represents not available.

Table 3. Characteristics of the variables including in the optimized logistic model

	Category	Cases(%) (n=153)	Controls(%) (n=190)	Coefficient	Significance (P value)	Odds Ratio (95% CI)
Age	60-69 (0)	37(24.2)	48(25.3)			
	70-79 (1)	69(45.1)	114(60.0)	-0.28	0.315 *	0.76(0.44~1.31)
	>=80 (2)	47(30.7)	28(14.7)	0.893	0.009 +	2.44(1.25~4.79)
Smoking	Never (0)	74(48.4)	125(65.8)			
	Ex (1)	49(32.0)	46(24.2)	0.63	0.019	1.88(1.11~3.18)
	Current(2)	30(19.6)	19(10.0)	1.09	0.002	2.97(1.50~5.86)
rs3753394	CC (0)	21(13.7)	34(17.9)			
	CT (1)	54(35.3)	87(45.8)	0.089	0.80	1.09(0.54~2.20)
	TT (2)	78(51.0)	69(36.3)	0.822	0.024	2.28(1.11~4.66)
rs1061170	CC (0)	1(0.7)	0(0)			
	CT (1)	15(9.8)	13(6.8)	-3.88	0.77	0.021(0~6.5*10E9)
	TT (2)	137(89.5)	177(93.2)	-4.74	0.73	0.009(0~2.7*10E9)
rs800292 ++	AA (0)	13(8.5)	29(15.3)			
	AG (1)	51(33.3)	82(43.2)	0.261	0.512	1.30(0.59~2.84)
	GG (2)	89(58.2)	79(41.6)	0.913	0.020	2.49(1.15~5.37)
rs1329428 ++	TT (0)	14(9.2)	36(18.9)			
	CT (1)	57(37.3)	77(40.5)	0.632	0.092	1.88(0.90~3.92)
	CC (2)	82(53.6)	77(40.5)	0.984	0.008	2.68(1.29~5.52)

The logistic regression model was optimized using the backward stepwise approach. In this model, age, smoking status, single nucleotide polymorphysm (SNP) rs3753394 and rs1061170 were included. Missing data of smoking status was excluded from the model. Therefore, the model was built with 343 samples (153 cases and 190 controls) with full data set. Asterisk (*) represents the p value obtained from the model was comparing category 1 with baseline (category 0) in cases and controls. Vertical arrow (+) represents the p value obtained from the model was comparing category 2 with baseline (category 0) in cases and controls. Double plus symbol (++) represents the SNPs rs800292 and rs1329428 were not included in the optimized model by backward stepwise approach. Here shows the coefficients and odds ratios in other models using "Enter" approach, adjusted for age, smoking and rs1061170.

sociated with exudative AMD in Japanese, the G allele at rs800292 is a risk factor in Chinese. This difference may be due to genuine ethnic heterogeneity although demographic factors, difference in inclusion criteria or sampling bias has to be ruled out.

SNP rs3753394 has no association with AMD in two studies in Caucasians [11,12]. According to the Hapmap project, the minor allele frequency of this SNP is 0.29 in CEU population and 0.47 in Chinese. It is not in the same LD block with rs1329428 in Caucasians and may subsequently contribute less to the susceptibility of AMD. But in Chinese, rs3753394 is in LD with rs1329428, and it is located at the promoter which regulates expression of the CFH. This SNP could be one of the AMD susceptibility polymorphisms in *CFH* in Chinese.

It has been suggested that the lower prevalence of AMD in Asians than in Caucasians could be a consequence of the low frequency of Y402H variant in Asians (0.07 in Chinese and 0.08 in Japanese from Hapmap project) [14]. It has been reported that the prevalence of AMD in ethnic Chinese living in USA was 4.6%, compared to 5.4% in whites ($p<0.001$) [33]. This difference in AMD prevalence could be partly due to the difference in the frequency of the Y402H variant between these two populations. The recessive pattern of association could explain in part such difference because carriers with heterozygous genotypes could have a relatively lower risk of AMD compared with the homozygous genotype carriers. However, Klein et al. has reported that the frequency of exudative AMD in Chinese was actually higher than whites [33]. Our findings that the genotypes of rs3753394 were significantly associated with exudative AMD susceptibility in Chinese population could in part explain this difference.

CFH is an important regulator of the complement system and an inhibitor of the alternative complement cascade. Impaired CFH function or expression may pose risk to diseases including hemolytic uremic syndrome (HUS), membranoproliferative glomerulonephritis (MPGN) and AMD [5,34]. CFH acts by binding C3b and as a cofactor in the proteolysis of C3b by factor I, resulting in an inactive C3b molecule [5,34]. The I62V variant is located at one of the regulatory domains for cofactor- and decay-accelerating activity and a C3b binding sites in the complement factor H [12,35] (Figure 1). Isoleucine and valine as branched chain amino acids are important for ligand binding to proteins and play central roles in protein stability [36]. Furthermore, I62V is located in a predicted exon splice enhancer [12,37]. Whether the substitution of an isoleucine by a valine may lead to splicing errors and result in alterations in CFH function that may affect the development of AMD remains to be investigated.

We for the first time detected a significant association between rs3753394 and exudative AMD. The risk TT genotype was present at a frequency of 50.9% in exudative AMD cases and 34.0% in controls, conferring a 2.28 (95% CI: 1.11-4.66) folds of increased risk adjusted for age, gender and smoking status. We found no other alterations in the promoter sequence up to -794 that was associated with AMD, indicating that rs3753394 is the only AMD polymorphism in this region of the CFH promoter. This SNP is located at -257 upstream in the *CFH* promoter between a glucocorticoid response element (GRE) at -232 and a possible histone H4 gene binding site, H4TF-1, which may be a common regulatory element, at position -273 [38] (Figure 1). It was also reported that the C-257T variant is located in a nuclear factor-kappa B (NFkB) responsive element within the HF1 promoter [39], which could play a role in regulating HF transcription.

Logistic regression analysis showed that older age was significantly associated with the susceptibility of exudative AMD. Aging had been reported to be associated with increased plasma level of factor H [40]. The increase in plasma CFH level should confer a certain reduced risk of AMD. However, AMD increases drastically with age. This may attribute to the fact that protection from the increased plasma levels of factor H in some individuals is not sufficient to compensate the risks from other age-related events, like cumulative oxidative injury [5]. Impaired CFH expression due to genetic factors may confer additional risk. In our study population, the T allele at rs3753394 confers a significantly increased likelihood of exu-

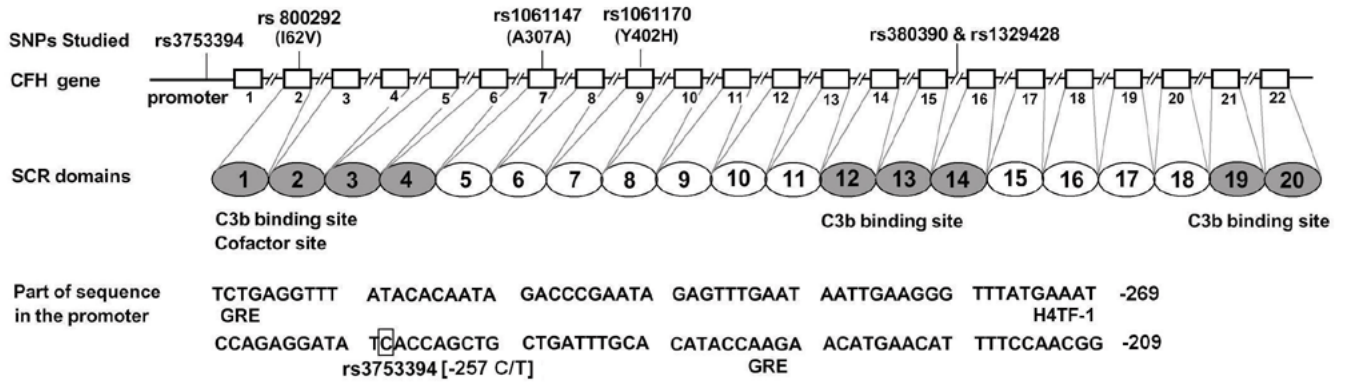

Figure 1. A schematic diagram of the *CFH* gene. Approximate locations of the 6 single nucleotide polymorphysms (SNPs) are shown on the top. The cofactor site for factor I and C3b binding sites are highlighted with grey color. Part of the sequence of the CFH promoter of the gene is shown at the bottom. Sequence elements and the SNP rs3753394 are shown below the corresponding sequences. SCR represents short consensus repeat, GRE represents glucocorticoid response element, H4TF-1 represents histone H4 gene binding site.

dative AMD. It is possible that the function of the promoter is impaired by the T allele and subsequently leads to relatively lower plasma levels of factor H. Further genotype-phenotype correlation studies should be performed to investigate the correlation between the genotypes at rs3753394 and the plasma levels of factor H in case-control studies in different age groups. Meanwhile, in our study, 34.0% of subjects carrying the TT genotype at rs3753394 did not develop exudative AMD. Other factors, genetic or environmental, likely play interactive or even protective roles. Recently, variants L9H and R32Q in the factor B (*FB*) gene and E318D in the complement component 2 (*C2*) gene have been shown to confer a significantly reduced risk of AMD [26].

In summary, our results showed that 3 SNPs of the *CFH* gene, rs1329428 and rs800292 (I62V) and for the first time rs3753394, but not rs1061170 (Y402H), conferred a significantly increased risk for exudative AMD in the Chinese population.

ACKNOWLEDGEMENTS

We express our greatest appreciation to all the participants in the study. This study is supported by a block grant of the Chinese University of Hong Kong and the Li Ka Shing Foundation Eye Research and Training Fund.

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The print version of this article was created on 5 Dec 2006. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.