

Multiple Gene Polymorphisms in the Complement Factor H Gene Are Associated with Exudative Age-Related Macular Degeneration in Chinese

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PURPOSE. Variants in the complement factor H (*CFH*) gene have been shown to be strongly associated with age-related macular degeneration (AMD). In this study, sequence alterations in *CFH* were investigated in 163 Chinese patients with exudative AMD and 155 unrelated Chinese control subjects.

METHODS. All the 22 *CFH* exons, intron-exon boundaries, and promoter sequences were screened by polymerase chain reaction and DNA sequencing.

RESULTS. Fifty-eight sequence changes, 42 of them novel, were identified. Six SNPs with an allele frequency >30% were significantly associated with exudative AMD. SNP rs3753396 was novel; the rest had been reported: rs3753394, rs551397, rs800292, rs2274700, and rs1329428. Two haplotype blocks were constructed. The TG haplotype for rs551397 and rs800292 was the major haplotype that conferred a significantly increased susceptibility to exudative AMD ($P_{\text{corr}} = 0.0001$, OR = 1.91, 95% CI = 1.36–2.68).

CONCLUSIONS. The findings support prior evidence that the *CFH* gene is one of the AMD-associated genes. There is a different distribution pattern of *CFH* variants in the Chinese compared with other populations. Individual SNP and haplotype analyses revealed that the ancient alleles at the 5' end of *CFH* contribute to an increased susceptibility to exudative AMD. (*Invest Ophthalmol Vis Sci.* 2008;49:3312–3317) DOI:10.1167/iovs.07-1517

Age-related macular degeneration (AMD; MIM 603075; Mendelian Inheritance in Man) is a major cause of irreversible visual impairment and blindness in people older than 65 years in developed countries.^{1,2} The occurrence of AMD is pan

ethnic, and a high prevalence AMD has been reported in the elderly Chinese population.^{3,4} Geographic atrophy (dry form) and exudative AMD (wet form) are severe forms of AMD, with the latter responsible for nearly 90% of patients with AMD who have inexorable and quick disease progression. Therapies such as photodynamic therapy and pharmacotherapy targeted at the overexpression of vascular endothelial growth factor have been shown to be effective in slowing down or even stopping vision loss. However, treatments that are effective in restoring visual acuity remain limited.⁵ Therefore, a greater understanding of the primary pathophysiology is needed to advance treatment and preventive measures.

The etiology of AMD is complex and multifactorial, probably resulting from interactions between environmental and multigenetic factors.⁶ Genetic association studies have revealed that single nucleotide polymorphisms (SNPs) in the complement factor H gene (*CFH*; MIM 134370; e.g., Tyr402His) are significantly associated with susceptibility to AMD.^{7–16} Polymorphisms in complement factor B (*CFB*, MIM 138470; e.g., Leu9His, Arg32Gln) and complement component 2 (*C2*, MIM 217000; e.g., Glu318Asp) also confer increased or decreased susceptibility to AMD.¹⁷ These genes encode regulatory proteins of the alternative complement pathway, supporting the proposition that innate immunity, the complement system, and the inflammatory pathway play major roles in the pathogenesis of AMD.¹⁸ Recently, SNPs in the hypothetical gene *LOC387715* (MIM 611313; e.g., Ala69Ser) and high temperature requirement factor A1 (*HTRA1*, MIM 602194; e.g., –635G>A) within the 10q26 locus have been found to be significantly associated with exudative AMD in Caucasians,^{19,20} Chinese,²¹ and Japanese,^{22,23} indicating that AMD pathogenesis could involve other pathways independent of the complements.²⁴

Recently, *CFH* haplotypes without the Tyr402His coding variant have been shown to confer strong AMD susceptibility in Caucasians.²⁵ A fine-scale linkage disequilibrium mapping of AMD in the *CFH* region detected a point location of a causal variant between exons 1 and 2 of *CFH* other than exon 9 for Tyr402His.²⁶ In our studies in Chinese patients, although variants in the *HTRA1* gene have been found to be strongly associated with exudative AMD,²¹ we also found AMD-associated *CFH* SNPs, including rs3753394 in the promoter, rs800292 (Val62Ile) in exon 2, and rs1329428 in intron 15, but not rs1061170 (Tyr402His) in exon 9.²⁷ In the present study, we screened the whole *CFH* gene, including all the coding sequences, exon-intron boundaries, and the promoter sequence in a cohort of Chinese study subjects.

MATERIALS AND METHODS

Patients and Control Subjects

All study subjects were ethnic Chinese recruited at the Eye Clinic of the Prince of Wales Hospital, Hong Kong. All underwent a standard

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Submitted for publication November 27, 2007; revised March 3, 2008; accepted June 16, 2008.

Disclosure: T.K. Ng, None; L.J. Chen, None; D.T.L. Liu, None; P.O.S. Tam, None; W.M. Chan, None; K. Liu, None; Y.J. Hu, None; K.K.L. Chong, None; C.S.L. Lau, None; S.W.Y. Chiang, None; D.S.C. Lam, None; C.P. Pang, None

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ophthalmic examination protocol. Grading was based on the standard classification described by the International Age-Related Maculopathy Epidemiologic Study Group.²⁸ In total, 163 patients with exudative AMD were recruited: 88 men and 75 women. The age at diagnosis ranged from 60 to 94 years (mean \pm SD, 75.5 \pm 7.5 years). All had been studied for the *CFH* genotype,²⁷ and among them 96 had been included in a published association study.²¹ Also recruited and given complete ophthalmic examinations were 155 unrelated control subjects, 72 men and 83 women ranging in age at recruitment from 60 to 99 years (mean \pm SD, 73.1 \pm 6.5 years). They matched the patients by age and gender and had no sign of AMD or other eye diseases, except mild myopia or senile cataract. 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. Informed consent was obtained from all study subjects after explanation of the nature of the study.

Sample Collection, PCR Amplification, DNA Sequencing, and SNP Genotyping

Venous blood was obtained from each study subject, and genomic DNA was extracted with a DNA blood kit (QIAamp; Qiagen, Hilden, Germany). The promoter sequence up to -867 upstream and all coding sequences of the *CFH* gene, including intron-exon boundaries, were screened for sequence alterations. Primers were generated based on the GenBank sequence of *CFH* (NM_000186.2; <http://www.ncbi.nlm.nih.gov/Genbank>; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). PCR was performed on a thermal cycler (model 9700; Applied Biosystems, Inc. [ABI], Foster City, CA) with optimized protocols (Supplementary Table S1 online at <http://www.iovs.org/cgi/content/full/49/8/3312/DC1>). Sequencing reactions were performed (BigDye Terminator Cycle Sequencing Kit, Ver. 3.1; ABI) according to the manufacturer's instructions and were analyzed on an automated DNA sequencer (model 3130XL; ABI). Sequence data were analyzed on computer (Chromas ver. 2.13; Technelysium Pty Ltd., Tewantin, QLD, Australia). SNP rs1329428 in intron 15 was genotyped in all samples (Prism 7000 Sequence Detection System; ABI) with a 5' allelic discrimination assay (TaqMan; ABI), according to the manufacturer's instructions, as previously described.²⁷

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) for each polymorphism was tested by χ^2 test. Allele or genotype frequencies between cases and control subjects were compared by χ^2 analysis or the Fisher exact test. The odds ratios (ORs) of the alleles and haplotypes were estimated by χ^2 test (SPSS ver.15.0; SPSS Inc., Chicago, IL). Population attributable risk (PAR) of the risk genotype was calculated with the formula $f(R - 1)/R$, where f is the fraction of cases with the risk genotype and R is the measure of OR⁸. A pair-wise linkage disequilibrium (LD, D') estimation between polymorphisms with a minor allele frequency (MAF) > 1%, and EM-based haplotype association analysis were performed with Haploview (ver. 3.32, from <http://www.broad.mit.edu/mpg/haploview/> provided in the public domain by the Broad Institute, Massachusetts Institute of Technology, Cambridge, MA). For multiple comparison, probabilities were corrected by permutation test (iterations, 10,000). Statistical significance was defined as a corrected P (P_{corr}) < 0.05.

RESULTS

CFH Variants in the Study Subjects

A total of 58 sequence variations were identified, all of which followed Hardy Weinberg Equilibrium (Table 1). Twenty-three had a MAF greater than 1% and were defined as SNPs. The remaining 35 were rare variants with MAF < 1%. There were 19 missense changes, 5 synonymous codon changes, and 34

changes in noncoding sequences, including 1 insertion and 2 deletions. Among them, 42 sequence changes were novel and have not been registered in an SNP database (dbSNP; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, available at www.ncbi.nlm.nih.gov/projects/SNP/).

Six of the seven common variants (Table 2) with MAF > 30% were significantly associated with exudative AMD: rs3753394, rs551397, rs800292, rs2274700, rs3753396, and rs1329428. The missense variant rs800292 (Val62Ile) in exon 2 and intronic variant rs551397 (IVS1-36C>T) had the strongest association with AMD, both with P_{corr} = 0.0001 and OR = 1.91. Association with AMD was newly discovered in this study for rs3753396. SNP rs1065489 (Asp936Glu) in exon 18 was the only common variant that was not associated with AMD (P_{corr} = 0.11; Table 2).

Six SNPs were identified in the promoter, all supported decreased susceptibility to AMD (Table 1), two of them with statistical significance: rs3753394 and rs35836460 (MAF_{case} = 0.9% and MAF_{control} = 3.9%, χ^2 test P = 0.014, OR = 0.23, 95% CI = 0.064 - 0.83). The novel SNP -261G>C was found in four control subjects, but not in patients. The only noncoding SNP outside the promoter that was significantly associated with AMD was located in intron 18 (rs16840522, IVS18-89T>C, MAF_{case} = 1.2% and MAF_{control} = 3.9%, χ^2 test P = 0.033, OR = 0.31, 95% CI = 0.098 - 0.97). Among the rare variants, 14 occurred exclusively in patients and 11 in control subjects, all were heterozygous. None of them showed significant association with AMD, probably because of their low frequencies. The other rare variants (including one insertion and two deletions) were evenly distributed in patients and control subjects (Table 1).

Haplotype Association Analysis

LD analysis revealed extension of LD throughout the *CFH* gene. We included SNPs with MAF > 5% and two missense changes, rs1061170 (Tyr402His) and Val837Ile, in our haplotype association analysis. Two distinct haplotype blocks were detected (Fig. 1). Block 1 included the two SNPs that were most significantly associated with the disease: rs551397 (IVS1-36C>T) and rs800292 (Val62Ile). Block 2 contained three AMD associated SNPs, rs2274700 (Ala473Ala), rs3753396 (Gln672Gln), and rs1329428. We listed the haplotypes constructed by all the common SNPs (MAF > 30%) and two missense variants (Tyr402His, Val837Ile; Table 3). B1h1 (block 1 haplotype 1) and B1h2 were strongly associated with AMD (P_{corr} = 0.0001). B1h1 conferred a 1.91-fold increased risk of exudative AMD, whereas B1h2 was protective. In block 2, B2h1 conferred a 1.75-fold significantly reduced risk of the disease (P_{corr} = 0.0024), whereas B2h2 conferred a 1.53-fold increased susceptibility (P_{corr} = 0.03).

The haplotypes H3 and H4, which were defined by all six AMD-associated SNPs, conferred significantly reduced or increased AMD susceptibility (H3: OR = 0.56, 95% CI = 0.39 - 0.80; H4: OR = 1.63, 95% CI = 1.19 - 2.23). When a G allele of rs1065489 (Asp936Glu) was included in these two haplotypes, the H5, which contained all the alleles in H3, remain significantly associated with the disease (P_{corr} = 0.0012). However, when a G allele or a T allele was added to the H4, the newly constructed H6 and H7 were no longer AMD associated (P_{corr} = 0.052 and 0.177, respectively).

We constructed two-allele haplotypes by using rs800292 (Val62Ile) with the uncommon SNPs rs1061170 (Tyr402His) and Val837Ile, to investigate the effects of the minor variants. H10 and H11, containing a T allele of rs1061170 (Tyr402His), remained significantly associated with AMD. However, the haplotypes containing a C allele of rs1061170 (Tyr402His) were not associated with AMD (data not shown). Haplotypes

TABLE 1. *CFH* Variants Observed in 163 Patients with Exudative AMD and 155 Control Subjects

Location	dbSNP ID	Sequence Change	Codon Change	Genotype Frequency*		Association (P value)	Allele Frequency (%)		Association (P/P _{corr})†
				Case	Control		Case	Control	
Promoter	Novel	-674C>G	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.32)	NS
Promoter	Novel‡	-650A>G	—	0/3/160	0/8/147	NS	3 (0.9)	8 (2.6)	NS
Promoter	Novel	-482G>A	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Promoter	rs3753394‡	-331T>C	—	22/58/83	32/69/54	0.013	102 (31.3)	133 (42.9)	0.002/0.020
Promoter	Novel	-261G>C	—	0/0/163	0/4/151	NS	0 (0.0)	4 (1.29)	NS
Promoter	rs35836460‡	-195T>C	—	0/3/160	0/12/143	0.013	3 (0.9)	12 (3.9)	0.014/0.12
Intron 1	Novel	IVS1+95T>C	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 1	rs551397‡	IVS1-36C>T	—	13/55/95	22/76/57	0.00056	81 (24.8)	120 (38.7)	0.00017/0.0015
Intron 1	Novel	IVS1-34C>T	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 2	rs800292‡	184G>A	Val62Ile	13/55/95	22/76/57	0.00056	81 (24.8)	120 (38.7)	0.00017/0.0015
Intron 3	Novel	IVS3+9T>C	—	0/4/159	0/1/154	NS	4 (1.2)	1 (0.3)	NS
Intron 3	Novel‡	IVS3+88T>C	—	0/3/160	0/6/149	NS	3 (0.9)	6 (1.9)	NS
Intron 3	Novel	IVS3+175C>A	—	0/1/162	0/3/152	NS	1 (0.3)	3 (0.97)	NS
Intron 3	Novel	IVS3-125_-131del7	—	0/1/162	0/1/154	NS	1 (0.3)	1 (0.3)	NS
Intron 3	Novel	IVS3-63T>A	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 3	Novel	IVS3-61G>A	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 4	Novel‡	IVS4+57insT	—	0/3/160	0/8/147	NS	3 (0.9)	8 (2.6)	NS
Intron 4	Novel‡	IVS4+108delT	—	0/4/159	0/3/152	NS	4 (1.2)	3 (0.97)	NS
Intron 4	rs3766403	IVS4-44A>T	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 5	Novel	476G>C	Ser159Thr	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 5	Novel‡	IVS5+195C>T	—	0/2/161	0/8/147	NS	2 (0.6)	8 (2.6)	NS
Exon 6	Novel	647T>C	Ile216Thr	0/2/161	0/1/154	NS	2 (0.6)	1 (0.3)	NS
Exon 7	Novel	907C>T	Arg303Trp	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 7	rs1061147‡	921C>A	Ala307Ala	1/17/145	0/9/146	NS	19 (5.8)	9 (2.9)	NS
Intron 7	Novel	IVS7+25C>T	—	0/2/161	0/3/152	NS	2 (0.6)	3 (0.97)	NS
Intron 7	Novel	IVS7+54C>T	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 7	rs482934‡	IVS7-53T>G	—	1/17/145	0/9/146	NS	19 (5.8)	9 (2.9)	NS
Exon 9	rs1061170‡	1204T>C	Tyr402His	1/17/145	0/9/146	NS	19 (5.8)	9 (2.9)	NS
Exon 9	Novel	1310C>A	Ser437Tyr	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 9	Novel	1330C>T	Arg444Cys	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 9	Novel	IVS9+10C>A	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 9	Novel	IVS9-46G>T	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 10	rs2274700‡	1419G>A	Ala473Ala	16/57/90	27/66/62	0.015	89 (27.3)	120 (38.7)	0.002/0.02
Exon 10	Novel	1456G>C	Gly486Arg	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 10	Novel	IVS10+40T>C	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 10	Novel‡	IVS10+168A>G	—	0/2/161	0/8/147	NS	2 (0.6)	8 (2.6)	NS
Intron 10	rs203674‡	IVS10-98T>G	—	1/17/145	0/9/146	NS	19 (5.8)	9 (2.9)	NS
Exon 11	rs35453854	1652T>C	Ile551Thr	0/1/162	0/1/154	NS	1 (0.3)	1 (0.3)	NS
Exon 12	Novel	1735G>A	Val579Ile	0/2/161	0/0/155	NS	2 (0.6)	0 (0.0)	NS
Exon 12	Novel	1736T>C	Val579Ala	0/1/162	0/1/154	NS	1 (0.3)	1 (0.3)	NS
Exon 13	Novel‡	1935G>T	Thr645Thr	0/2/161	0/8/147	NS	2 (0.6)	8 (2.6)	NS
Exon 13	rs3753396‡	2016G>A	Gln672Gln	21/66/76	34/69/52	0.024	108 (33.1)	137 (44.2)	0.004/0.041
Exon 14	Novel	2089C>T	Leu697Phe	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Exon 14	Novel	2114C>T	Ser705Phe	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 14	Novel	IVS14-52T>C	—	0/0/163	0/2/153	NS	0 (0.0)	2 (0.6)	NS
Intron 15	Novel	IVS15+20T>G	—	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Intron 15	Novel	IVS15-32A>T	—	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 15	rs375046‡	IVS15-28A>C	—	4/14/145	0/12/143	NS	22 (6.7)	12 (3.9)	NS
Exon 16	Novel‡	2509G>A	Val837Ile	0/2/161	0/8/147	NS	2 (0.6)	8 (2.6)	NS
Exon 17	Novel‡	2637A>G	Gly879Gly	0/2/161	0/8/147	NS	2 (0.3)	8 (2.6)	NS
Exon 17	Novel	2669G>C	Ser890Thr	0/0/163	0/1/154	NS	0 (0.0)	1 (0.3)	NS
Exon 18	rs1065489‡	2808T>G	Asp936Glu	23/67/73	34/69/52	0.064	113 (34.7)	137 (44.2)	0.014/0.11
Exon 18	Novel	2944C>T	Pro982Ser	0/1/162	0/0/155	NS	1 (0.3)	0 (0.0)	NS
Intron 18	rs16840522‡	IVS18-89T>C	—	0/4/159	0/12/143	0.031	4 (1.2)	12 (3.9)	0.033/0.26
Intron 19	Novel‡	IVS19+8G>T	—	0/6/157	0/5/150	NS	6 (1.8)	5 (1.6)	NS
Exon 20	Novel	3172T>C	Tyr1058His	0/2/161	0/1/154	NS	2 (0.6)	1 (0.3)	NS
Exon 20	rs410232	3178G>C	Val1060Leu	0/2/161	0/1/154	NS	2 (0.6)	1 (0.3)	NS
Exon 22	Novel	3696+99G>A	—	0/2/161	0/1/154	NS	2 (0.6)	1 (0.3)	NS

Reference sequence: GenBank sequence of the *CFH* gene (NM_000186.2).

* The numbers indicate homozygous variant genotype/heterozygous genotype/homozygous reference genotype.

† The probabilities of allelic association estimated by χ^2 test and the corrected probabilities (P_{corr}) estimated by permutation test (iterations, 10,000) are shown.

‡ Variant with an allele frequency greater than 1%, which is defined as SNP.

TABLE 2. Association between Common *CFH* Polymorphisms and Exudative AMD

Polymorphism	db SNP ID	Codon Change	Risk Allele Frequency (%)			Association (P/P_{corr})*	OR (95% CI)
			Allele	Case	Control		
-331T>C	rs3753394	—	T	68.7	57.1	0.002/0.020	1.65 (1.19-2.28)
IVS1-36C>T	rs551397	—	C	75.2	61.3	0.00017/0.0015	1.91 (1.36-2.68)
184G>A	rs800292	Val62Ile	G	75.2	61.3	0.00017/0.0015	1.91 (1.36-2.68)
1419G>A	rs2274700	Ala473Ala	G	72.7	61.3	0.002/0.020	1.68 (1.20-2.35)
2016G>A	rs3753396	Gln672Gln	G	66.9	55.8	0.004/0.041	1.60 (1.16-2.20)
IVS15-3109C>T	rs1329428	—	C	72.7	58.7	0.0002/0.0024	1.87 (1.34-2.61)
2808T>G	rs1065489	Asp936Glu	T	65.3	55.8	0.014/0.11	1.49 (1.08-2.06)

A common polymorphism was defined as a polymorphism with MAF > 30%.

* Probabilities for allelic association from χ^2 test and corrected probabilities from a permutation test.

H12 and H13 containing a G allele rather than the A allele of SNP Val837Ile were significantly associated with AMD. These results indicate that the minor alleles in these missense SNPs add little to AMD susceptibility.

DISCUSSION

Although the pathogenesis of exudative AMD has not been definitively elucidated, studies in the past few years have revealed important information on its genetic basis. Polymorphisms in the *CFH* gene have been shown to be AMD associated in different ethnic groups, although there are obvious differences in the occurrence of disease-susceptible SNPs between Caucasian and Oriental populations.^{7-16,30} Results of this study in Chinese individuals provide additional evidence that *CFH* is a genetic risk factor for AMD. We found two haplotype blocks. The first is located in the 5' region covering intron 1 (rs551397) and exon 2 (rs800292), and the second is in the middle region covering exon 10 (rs2274700) to intron 15 (rs1329428). The haplotypes defined by the major alleles of the SNPs in the two haplotype blocks conferred a 1.91 (B1h1)-

and 1.53 (B2h2)-fold increased susceptibility to exudative AMD, whereas the haplotypes defined by the minor alleles conferred a reduced risk. SNP Val62Ile in block 1 encoded for a missense amino acid change, whereas the SNPs in block 2 were all synonymous changes. The OR of B1h1 was greater than that of B2h2, although the CIs of two haplotypes were partly overlapped. Based on these findings, we hypothesize that block 1 could be the major region in the *CFH* which is associated with exudative AMD in our cohort, and block 2 confers a milder effect. Homozygosity (GG) for Val62Ile was present in 58.3% of cases and 36.8% of controls, conferring a 2.82 (95% CI = 1.32-6.03)-fold increased risk of exudative AMD compared with the AA genotype. The PAR for GG genotype was 37.6% (95% CI = 14.1%-48.6%). In contrast, the heterozygous genotype (GA) was not significantly associated with the disease, suggesting that an allelic dosage effect exists in the association between this SNP and AMD. Recently, Ennis et al.²⁶ mapped a point location for a causal variant between exons 1 and 2, which approximates block 1 in our present study, suggesting that the 5' region of the *CFH* (N-terminal of factor H) is commonly associated with AMD in both Chinese and Caucasians.

We found haplotype block 2 spanning a region from exon 10 to intron 15 and containing SNP rs2274700 (Ala473Ala, exon 10), which have recently been shown to have a strong association with AMD in Caucasians and Japanese.^{31,32} Block 2 also included SNP rs3753396 (Gln672Gln, exon 13), which was not significantly associated with AMD in Caucasians,¹⁰ but for the first time was found to be associated with exudative AMD in the Chinese in this study.

Besides the haplotypes in the two haplotype blocks, the haplotypes defined by the six common SNPs (H3, H4) were also significantly associated with exudative AMD. However, when Asp936Glu (in exon 18) was included in the at-risk haplotype H4 for association analysis, the haplotypes H6 and H7, including a G or a T allele respectively, were no longer significantly associated with the disease ($P_{corr} > 0.05$). Thus, Asp936Glu is less likely to be a risk factor for exudative AMD in Chinese individuals, indicating the C-terminal of the factor H contributes less than other parts of the polypeptide to the development of exudative AMD. This observation is consistent with the findings of Hageman et al.¹⁰ in Caucasians.

Our results also confirmed differential occurrences of AMD-associated *CFH* polymorphisms, such as rs1061170 (Tyr402His) among Chinese, Caucasians, and even Japanese. We found that Tyr402His was present at low frequencies, with 5.8% in cases and 2.9% in controls, and was not associated with AMD. It was in complete linkage disequilibrium (LD, $D' = 1$) with the other three SNPs: rs1061147 (Ala307Ala, exon 7), rs482934 (IVS7-53T>G), and rs203674 (IVS10-98T>G). The relatively uncommon SNPs in this region are less likely to be the genetic risk factors of exudative

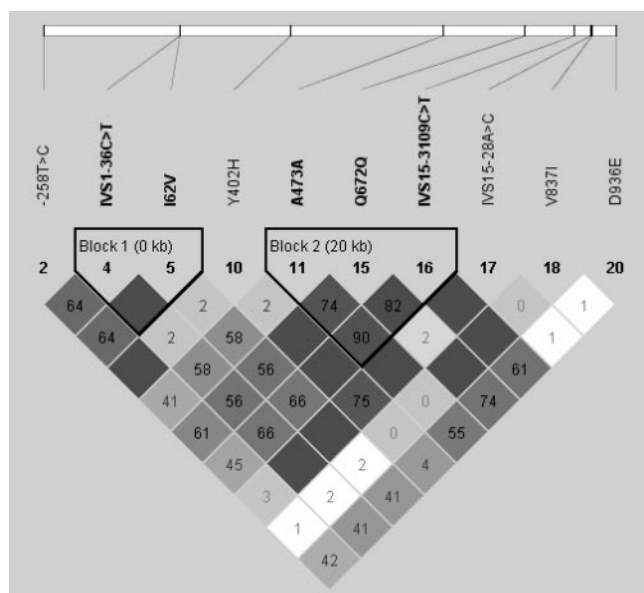


FIGURE 1. LD between the polymorphisms in the *CFH* gene. *CFH* polymorphisms with MAF > 5%, as well as two missense SNPs (Tyr402His and Val837Ile), were included in the analysis. Two distinct haplotype blocks were defined by the confidence intervals, an algorithm proposed by Gabriel et al.²⁹ using Haploview. The LD (r^2) between any two SNPs is listed in the cross cells.

TABLE 3. Haplotype Analysis of the CFH Polymorphisms between Cases and Controls

Order	Frequency											OR (95% CI)	
	rs3753394	rs551397	rs800292	rs1061170	rs2274700	rs3753396	rs1329428	2509 G > A	rs1065489	Case	Control		P _{corr}
B1h1	—	C	G	—	—	—	—	—	—	0.752	0.613	0.0001	1.91 (1.36–2.68)
B1h2	—	T	A	—	—	—	—	—	—	0.248	0.387	0.0001	0.52 (0.37–0.74)
B2h1	—	—	—	—	A	A	T	—	—	0.264	0.387	0.0024	0.57 (0.41–0.79)
B2h2	—	—	—	—	G	G	C	—	—	0.659	0.558	0.03	1.53 (1.11–2.11)
H3	C	T	A	—	A	A	T	—	—	0.204	0.316	0.0052	0.56 (0.39–0.80)
H4	T	C	G	—	G	G	C	—	—	0.624	0.505	0.0087	1.63 (1.19–2.23)
H5	C	T	A	—	A	A	T	—	G	0.182	0.300	0.0012	0.52 (0.36–0.76)
H6*	T	C	G	—	G	G	C	—	G	0.056	0.017	0.052	3.57 (1.31–9.72)
H7	T	C	G	—	G	G	C	—	T	0.568	0.487	0.177	1.38 (1.01–1.89)
H8	—	—	A	—	—	—	—	—	G	0.208	0.336	0.0011	0.52 (0.37–0.75)
H9	—	—	G	—	—	—	—	—	T	0.612	0.507	0.030	1.55 (1.13–2.12)
H10	—	—	A	—	—	—	—	—	—	0.248	0.387	0.0004	0.52 (0.37–0.74)
H11	—	—	G	—	—	—	—	—	—	0.693	0.584	0.014	1.61 (1.16–2.23)
H12	—	—	G	—	—	—	—	G	—	0.751	0.609	0.0004	1.94 (1.38–2.72)
H13	—	—	A	—	—	—	—	G	—	0.243	0.365	0.0015	0.56 (0.40–0.79)

All of the common SNPs (MAF > 30%) and two missense SNPs (Tyr402His, Val837Ile) were included in the haplotype association analysis. Only haplotypes with a frequency greater than 5% are shown. B1, haplotype block 1; B2, haplotype block 2; P_{corr} association analysis results from permutation test (iterations, 10,000); —, SNP that was not included in the haplotype association analysis. * Although the haplotype frequency of H6 in all subjects was less than 0.05, it is included because it was informative (P_{corr} = 0.052).

AMD in Chinese. However, in Caucasians, the SNPs Tyr402His and Ala307Ala were significantly associated with AMD.¹⁰ The C allele in Tyr402His, present in 34.9% of Caucasian population, was estimated to play a role in almost 60% of AMD at the population level.³³ In contrast, in a Japanese sample, the frequency of allele C in Tyr402His in two independent studies was 5.6% and 4.0% in control subjects,^{34,35} being mildly but not significantly higher than the 2.9% in our study (χ^2 test, $P > 0.05$). In a Taiwan Chinese population, a C allele frequency, 2.8%, similar to that in our control subjects was reported.³⁰ However, the C allele in their AMD patients was much higher in frequency, 11.3%, when compared with the 5.8% in our study (11.3% vs. 5.8%, χ^2 test, $P = 0.012$). Such discrepancy in the distribution of the Tyr402His variant between Chinese in Hong Kong and in Taiwan could be due to stratification of the population. Further investigations are needed, especially a replication of the Taiwan study.

We also found that all the risk alleles in *CFH* polymorphisms were the major alleles (ancient alleles). Therefore, ancient alleles could predispose Chinese to exudative AMD. In contrast, the so-called “protective” minor alleles may confer reduced genetic susceptibility to AMD during human evolution. However, up to 8.0% of our patients were homozygous for the protective haplotype B1h2 (TA), and 36.8% of the control subjects possessed the homozygous risk haplotype B1h1 (CG). Therefore, the development of exudative AMD should have resulted from interactive effects of the *CFH* gene with multiple genetic and environmental risk factors.

SNP rs11200638 (–625G>A) in the promoter of *HTRA1* has been reported to be a major genetic risk factor for exudative AMD.^{20,21} We found that the at-risk genotype AA of rs11200638 in the *HTRA1* promoter conferred a stronger susceptibility for exudative AMD than did the genotype GG of the *CFH* SNP Val62Ile in our cohort (data not shown). No interaction was found between these two polymorphisms; only an additive effect was detected (combined PAR = 74.8% for the homozygous at-risk genotypes at these two SNPs). Similar results were also suggested by other studies.³⁶ Recently, Kanda et al.³⁷ reported that SNP rs10490924 of the *LOC387715/ARMS2* gene is strongly associated with AMD and contributes much higher susceptibility to the development of AMD than does *HTRA1*. *LOC387715/ARMS2* codes for a mitochondrial protein. Their results suggest the involvement of mitochondrial oxidation reduction in AMD pathogenesis.

Dinu et al.³⁸ have identified two additional genes, *C7* (MIM 217070) and *MBL2* (MIM 154545), involved in the complement pathway that has been significantly associated with AMD. The complement C3 (MIM 120700) variant rs2230199 has also been found to be strongly associated with AMD in Caucasians.³⁹ These findings provided further evidence that the complement pathway plays a role in AMD pathogenesis. Further investigations of the roles of these genes (*CFB*, *C2*, *C3*, and *C7*) and the gene-gene interactions among them with *HTRA1* and *LOC387715/ARMS2* would be worthwhile.

Acknowledgments

The authors thank all who participated in the study.

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