A Prospective Assessment of the Y402H Variant in Complement Factor H, Genetic Variants in C-Reactive Protein, and Risk of Age-Related Macular Degeneration

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Purpose. Two biologically related factors, complement factor H (CFH) and C-reactive protein (CRP), have been associated with AMD. The Y402H variant of CFH is located within the binding site of CFH for CRP. Although plasma CRP levels have been related to AMD and plasma CRP levels are partly determined by genetic variation, there is no information on whether genetic variants in *CRP* are associated with AMD.

Methods. A prospective analysis was performed of 111 men who eventually developed AMD and 401 men who remained free of AMD, all participants in the Physicians' Health Study. Genotypes were determined for the common T→C single nucleotide polymorphism (SNP) in exon 9 of *CFH* (rs1061170; protein Y402H), as well as seven previously described *CRP* SNPs (rs3093059, rs2794521, rs3091244, rs1417938, rs1800947, rs1130864, and rs1205). Logistic regression analysis was used to evaluate individual SNPs, as well as six *CRP* haplotypes for association with AMD.

Results. The high-risk C allele of *CFH* was present in 45% of cases and 34% of controls. An odds ratio (OR) of 1.46 was observed for AMD (95% confidence interval [CI]: 1.05–2.04) for TC heterozygotes and an OR of 2.13 (95% CI: 1.10–4.16) for CC homozygotes, assuming a multiplicative (log-additive) model and attributable fraction of 25% (95% CI: 1% to 44%) was calculated. For *CRP*, single-marker or haplotype-based analysis failed to reveal any significant associations with a risk of AMD.

Conclusions. These prospective data confirmed an association between the Y402H variant of *CFH* and a risk of AMD. In contrast, although a biologically plausible, genetic variation in *CRP* does not appear to be associated with a risk of AMD. Further prospective studies of a larger number of subjects are needed to substantiate available information on the genetic epidemiology of AMD. (*Invest Ophthalmol Vis Sci.* 2006;47: 2336–2340) DOI:10.1167/iovs.05-1456

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Recent work[1] suggests that aging may be intimately related to chronic low-grade inflammation, resulting from the body's response to accumulated stress from myriad stressors, such as UV radiation, oxidation, and infection. This chronic stress leads to the development, at varying rates among individuals, of a subclinical, chronic inflammatory response.[1] The level of this inflammatory response predicts the development of several age-related degenerative diseases,[2] including AMD,[3] the leading cause of blindness and visual impairment among aging populations.

Consistent with a pathogenic role for inflammation, recent work has identified a strong association between a common variant of the gene for complement factor H (*CFH*) (dbSNP: rs1061170, sequence: T1277C, protein: Y402H) and the risk of AMD.[4–10] This association is plausibly causative, because the *CFH* gene maps to a location on the long arm of chromosome 1, termed the *ARMD1* locus, which was linked with AMD in several studies[11]; relevant biological mechanisms can be theorized; and its significance has now been consistently replicated in a number of studies.[4–10] However, the magnitude of prior estimates, with relative risks in the range of 2.5 to 7.4, must be interpreted in light of the study design and the selected nature of cases, as well as controls, including, for example, clinic-based ascertainment of prevalent cases in most instances as well as the restriction in one study that control subjects must have no family history of AMD,[6] all of which might bias risk estimates upward.[12] Moreover, although *CFH* (or a tightly linked gene) appears to play a role in AMD, the high prevalence of the risk allele in the population suggests a stochastic effect or the existence of interacting risk factors.

In addition, plasma levels of C-reactive protein (CRP), an acute-phase reactant and systemic marker of subclinical inflammation, have been found to be associated with the risk of AMD.[13,14] CFH interacts with high affinity to CRP to reduce deposition of the terminal attack complex of complement (C5b-9), and the presumed causal Y402H variant of CFH is thought to lead to changes in the activities of the binding sites on CFH for CRP that could alter the ability of CFH to suppress complement-mediated damage.[15] Recent work has shown that variation in plasma CRP is at least partly determined by genetic variation in the gene for CRP.[16,17] However, there is no information on whether genetic variants in *CRP* are associated with AMD.

We therefore investigated the association between the Y402H variant in CFH and seven common variants in *CRP* and AMD in a prospective study of a subset of subjects from the Physicians' Health Study (PHS), a cohort of 22,071 initially healthy male physicians.

Materials and Methods

Study Population

We conducted a prospective study of a subset of participants in the PHS cohort, a randomized, double-masked, placebo-controlled trial of

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aspirin and β-carotene for the primary prevention of cardiovascular disease and cancer among 22,071 U.S. male physicians that began in 1982 and has been conducted in accordance with the Declaration of Helsinki. At baseline, 14,916 PHS participants provided blood samples. Among these subjects, 1712 were selected for participation in a nested case–control study of the genetic epidemiology of cardiovascular disease. Each cardiovascular disease case was matched to one control subject chosen randomly from participating physicians who reported no cardiovascular disease at the time the case participant reported the event and who met matching criteria of age, smoking habit, and time since randomization. For the present study, we excluded 88 nonwhite men, 69 men for whom relevant genotype data were not available, 17 men who reported a diagnosis of AMD before baseline, and 27 men with a diagnosis of deep vein thrombosis. Among the remaining men, we identified 111 white participants with a confirmed diagnosis of incident AMD. For the control group, we excluded 999 men who were ≤65 years old, leaving 401 white men available to serve as control subjects. The availability of CFH and CRP genotype data among this subset of PHS participants provided a cost-effective means of investigating whether variants of these genes are associated with AMD.

Ascertainment of AMD Cases

Procedures for documentation of incident cases of AMD have been described previously.[18] Beginning with the 7-year follow-up questionnaire, we asked PHS participants about the diagnosis of AMD and updated this information on all subsequent annual follow-up questionnaires. We requested the month and year of diagnosis, the name and address of the diagnosing eye doctor, and a signed permission to review medical records. On receipt of permission, we sent a letter to the participant's eye doctor that contained a brief questionnaire to obtain information on the date of diagnosis, the best corrected visual acuity at the time of diagnosis, and the chorioretinal lesions that were observed at diagnosis (drusen; RPE changes, including atrophy, hypertrophy, and RPE detachment; geographic atrophy; subretinal neovascular membrane; disciform scar). For the present study, we included all confirmed cases of AMD whether or not visual acuity was affected.

To validate our methodology for AMD case ascertainment, we performed a validation study in the Nurses' Health Study (NHS), in which we used parallel methodology for confirmation of AMD. Briefly, we requested 30° color stereo photographs from cases reported on the 1990, 1992, and 1994 NHS questionnaires and subsequently confirmed by independent review of medical record information to meet our case definition. Two experienced retinal specialists with specific expertise in research on macular degeneration reviewed the slides by using a magnified (4×) stereo viewe to evaluate a circle with a radius of two disc diameters from the center of the fovea. We reviewed slides from 180 of a possible 210 eligible cases. Among these, we received 143 slide sets that were of sufficient quality to grade, 132 (92%) of participants that were judged to have definite AMD, and 5 (3%) of participants with probable AMD. Thus, 95% of medical-record–confirmed cases of AMD were considered definite or probable AMD on independent review of fundus photographs.

SNP Genotyping

CFH genotypes were determined by using a fluorescence-based detection method (Assay-by-Design; Applied Biosystems [ABI], Foster City, CA). Seven *CRP* SNPs were genotyped by either a matrix-assisted laser desorption (Sequenom, San Diego, CA) and matrix-assisted laser desorption ionization mass spectrometry–time-of-flight (MALDI-TOF) mass spectrometry or assays (Prism *Taq*Man; ABI) according to standard protocols, as previously described.[16] To confirm genotype assignments, we performed genotyping in duplicates blinded as to case–control status, and two independent observers performed the scoring. Disagreements (<1% of all scoring) were resolved by a joint reading and, if necessary, repeat genotyping. The *CRP* SNPs included a T→C substitution at −757 (rs3093059), at C→T substitution at −717 (rs2794521), a triallelic C→T/A substitution at −286 (rs3091244), a T→A substitution at 349 (rs1417938), a G→C substitution at 1059 (rs1800947), a C→T substitution at 1444 (rs1130864), and a G→A substitution at 1846 (rs1205).

Statistical Analysis

Genotype and allele frequencies between cases and controls were compared by using the χ^2 analysis. Tests for Hardy-Weinberg equilibrium (HWE) were performed by χ^2 analysis. Pair-wise linkage disequilibrium (LD) was examined as described by Devlin and Risch.[19] We used logistic regression models to estimate the odds ratios (OR) and corresponding 95% confidence intervals (CI) for the effect of genotypes on risk of AMD adjusted for other risk factors. Haplotype frequencies were estimated from genotype data by using (Phase ver. 2.1),[20,21] and the haplotype distribution between cases and controls was compared by a likelihood ratio test. In addition, we examined the relation between haplotypes and AMD outcome by logistic regression analysis by using a baseline-parameterization approach,[22] adjusting for age, smoking, body mass index, diabetes, prior myocardial infarction or stroke, hypertension, and randomized treatment group. A two-tailed $P \leq 0.05$ was considered to represent a statistically significant result. All analyses were performed on computer (SAS ver. 9.1/Genetics; SAS, Cary, NC). For analysis of single *CRP* SNPs, the study had 80% power to detect ORs ranging from 1.8 for the most prevalent SNP to 2.2 for the least prevalent SNP.[23] Finally, we calculated the attributable fraction in the population and its 95% CI as a measure of the proportion of AMD associated with the *CFH* polymorphism.[24]

Results

Of the 111 participants with AMD, 72 had visual acuity of 20/30 or worse because of AMD, including 21 cases with the neovascular form of the disease, and 51 cases with the dry form. Control subjects were by design significantly older than the AMD cases (Table 1). Apart from control subjects also being more likely to have hypertension, the distribution of other factors, such as smoking status, body mass index, history of hyperlipidemia, diabetes mellitus, use of aspirin, and family history of premature coronary artery disease, were not different between AMD cases and controls. There was no evidence for departures from HWE for any of the SNPs in either cases or controls.

The high-risk C allele of *CFH* was present in 45% of cases and 34% of controls ($P = 0.005$), and the TC and CC genotypes were more common among cases as presented in Table 2 (P for trend = 0.008). In logistic regression models for all AMD cases and controls, we observed significant association of the *CFH* polymorphism with AMD (P for trend = 0.02), with an OR of 1.46 (95% CI: 1.05–2.04) for TC heterozygotes and 2.13 (95% CI: 1.10–4.16) for CC homozygotes, assuming an additive model (such that the effect of the number of risk alleles is additive on the log-odds scale) adjusted for age, smoking, body mass index, history of hypertension, presence or absence of diabetes, randomized aspirin and β-carotene treatment assignments, and cardiovascular disease status. In a similar model limited to subjects who had visual acuity of 20/30 or worse because of AMD, the OR (95% CI) estimates were 1.65 (1.12–2.42) for TC heterozygotes and 2.72 (1.25–5.86) for CC homozygotes. Based on these data, the population attributable risk for CFH Y402H was estimated to be 25% (95% CI: 1%–44%).

The estimated ORs were of similar magnitude among neovascular cases, though CIs were wide because of the small number of cases (data not shown). In additional analyses in which we included men ≤65 years old in the control group, estimates were also not different, apart from narrower CIs because of the increased sample size (data not shown).

TABLE 1. Baseline Characteristics of Study Participants

	Controls (n = 401)	AMD Cases (n = 111)	P Value
Age (y)	70.1 ± 0.2	65.4 ± 0.7	<0.0001
Smoking status (%)			0.32
Never	41.2	43.6	
Past	49.2	42.7	
Current	9.5	13.6	
Body mass index (kg/m^2)	24.8 ± 0.1	24.9 ± 0.3	0.71
Blood pressure (mm Hg)			
Systolic	134.7 ± 0.7	132.4 ± 1.4	0.13
Diastolic	81.1 ± 0.4	80.4 ± 0.8	0.45
Hyperlipidemia (%)	16.1	14.0	0.60
Hypertension (%)	50.8	35.5	0.004
Diabetes (%)	6.8	5.4	0.61
Aspirin use (%)	44.9	40.5	0.41
Family history of premature CAD (%)	5.4	8.2	0.28

Mean ± SE. CAD, coronary artery disease.

In marker-by-marker χ^2 analyses of the seven *CRP* SNPs (Table 3), none of the alleles or genotypes was significantly associated with AMD. Similarly, we did not observe any significant associations between the seven *CRP* SNPs and AMD in logistic regression models adjusted for age, smoking, and other factors, and assuming an additive model (Table 4). The *CRP* variants tested were in LD with each other as previously reported.[16] Haplotype frequencies ≥1% based on the seven genotyped *CRP* SNPs were not significantly different between cases and controls, and haplotype-based logistic regression analysis did not uncover any significant associations with a risk of AMD, although CIs were wide (data not shown).

DISCUSSION

Data derived from epidemiologic, genetic, laboratory, and pathologic studies support the theory that inflammation has a role in the initiation and progression of AMD. [3–6,13] In this prospective study of a subset of AMD cases and controls from the PHS, we observed a significant association of the Y402H variant of CFH and risk of AMD, confirming the results of prior studies.[4–10] In contrast, we did not observe any significant association between common genetic variants in *CRP* and a risk of AMD, though power for detection of modest associations was limited.

Participants in the present study were originally selected for a study of the genetic epidemiology of cardiovascular disease. However, any bias should be minimal, given the lack of association between the Y402H variant and cardiovascular disease in this population[25] and our control for these conditions in regression models. Assessment of AMD relied on self-reports confirmed by review of medical record information,[18,26,27] and some cases of AMD may therefore have been missed or misdiagnosed. However, in a cohort study, including in the case of nested case–control sampling from a cohort, a disease definition that has low sensitivity but high specificity does not bias the observed relative risk, because there is a proportional reduction of cases in each exposure group so long as case ascertainment is unrelated to the exposure of interest (in our study, the *CFH* and *CRP* genotypes).[28] Although we do not know the sensitivity of our case definition, our validation indicates high specificity.[29] Nonetheless, because subjects were not examined, it remains possible that associations were underestimated. The likelihood of underestimation may be increased given the lower prevalence of CFH Y402H variants observed among the cases in the present study compared with others.[4–8] Given the relatively small number of cases, this observation may be from chance, or possibly from the inclusion of a case group with milder forms of AMD.

TABLE 2. Complement Factor H Genotype and Allele Distribution

rs1061170 (Y402H)	Controls (n = 401)	AMD Cases (n = 111)	P Value
Genotype (%)			
TT	44.89	33.33	
TC	41.40	44.14	
CC	13.72	22.52	0.008*
T	0.66	0.55	
C	0.34	0.45	0.005

* P value for exact test for trend across genotypes. P value for the two degree of freedom exact test of homogeneity of genotype distribution among cases and controls is 0.03.

Chronic inflammation could influence the risk of AMD through various pathways, including endothelial dysfunction in choroidal vessels, development of basal deposit and drusen, and degeneration of Bruch's membrane. All of these potential points of attack would result in alterations in the transport and exchange of nutrients and waste materials between the RPE and the choroid and could cause the characteristic changes observed in AMD. Inflammatory stimuli are also known to increase the production of reactive oxygen intermediates, which are thought to play a key role in the pathogenesis of AMD.[30,31] Furthermore, inflammation can reduce the bioavailability of antioxidants, setting the stage for a vicious cycle of altered redox status and increased oxidative stress.[30]

The present findings confirm several prior reports that a common variant in *CFH* is strongly associated with a risk of AMD.[4–10] CFH plays an intimate role in the regulation of an alternative pathway of complement activation, and alterations in the ability of CFH to suppress complement-mediated damage among people with the Y402H variant of CFH could increase the level of subclinical inflammation and spur the progression of AMD.[3,32,33]

CFH interacts biologically with CRP, and CFH Y402H is located within the binding site on CFH for CRP.[15] CRP is a major acute-phase reactant produced by the liver. Plasma levels of CRP may remain slightly elevated in states of chronic low-level inflammation that occur in a subset of otherwise healthy individuals during aging. Thus, mildly elevated levels of CRP appear to be a useful marker of a heightened state of inflammation or nonspecific immune reactivity. Higher CRP levels have previously been found to be associated with the presence

and the progression of advanced AMD[13,14]; although not all investigators have observed a relation.[34–36] It is plausible that CRP has some direct pathophysiologic role in AMD, perhaps mediated through its capacity to induce complement activation via the alternative pathway and contribute to tissue damage through several complement-mediated mechanisms.[37] However, the biological functions of CRP have yet to be fully elucidated, and it remains possible that plasma CRP levels are a risk marker, perhaps of a heightened state of immune reactivity or inflammatory activity, with no direct biological role in AMD pathogenesis. The tri-allelic SNP located at −286 from the start of transcription recently was associated with higher plasma CRP levels among carriers of the rarer T and A alleles at this locus.[17,38] However, in the present study, we did not observe carriers of the rarer alleles to be more prone to development of AMD. It is possible that the association of variation within *CRP* with AMD is influenced by variation in *CFH* or other factors, a hypothesis that the present study was not adequately powered to investigate.

TABLE 3. C-Reactive Protein Genotype and Allele Distribution

Genotype (%) or Allele	Controls (n = 401)	AMD Cases (n = 111)	P Value*
rs3093059 (−757T→C)			
TT	86.90	90.11	
TC	12.80	9.89	
CC	0.30	0.00	0.68
T	0.93	0.95	
C	0.07	0.05	0.39
rs2794521 (−717C→T)			
CC	58.55	51.61	
CT	33.91	43.01	
TT	7.54	5.38	0.27
C	0.76	0.73	
T	0.24	0.27	0.50
rs3091244 (−286C→T→A)			
CC	36.97	44.44	
CT	37.88	35.56	
TT	8.79	5.56	
CA	11.82	8.89	
TA	4.24	4.44	
AA	0.30	1.11	0.55
C	0.60	0.65	
T	0.33	0.29	
A	0.07	0.06	0.52
rs1417938 (349T→A)			
TT	49.21	50.94	
TA	40.84	38.68	
AA	9.95	10.38	0.92
T	0.70	0.70	
A	0.30	0.30	0.86
rs1800947 (1059G→C)			
GG	86.78	86.49	
GC	12.72	13.51	
CC	0.50	0.00	0.92
G	0.93	0.93	
C	0.07	0.07	0.96
rs1130864 (1444C→T)			
CC	46.24	48.39	
CT	43.64	40.86	
TT	10.12	10.75	0.90
C	0.68	0.69	
T	0.32	0.31	0.84
rs1205 (1846G→A)			
GG	42.77	42.86	
GA	44.28	43.96	
AA	12.95	13.19	1.00
G	0.65	0.65	
A	0.35	0.35	0.99

All genotype distributions in Hardy-Weinberg equilibrium.

* P value for exact test of genotype distribution or χ^2 test of allele distribution.

TABLE 4. Single-Marker Logistic Regression Analyses for the Variants Evaluated and Risk of AMD

Additive Mode	OR*	95% CI*	P Value
CFH rs1061170 (Y402H)	1.46	1.05–2.04	0.02
CRP			
rs3093059 (−757T→C)	0.66	0.29–1.54	0.34
rs2794521 (−717C→T)	1.03	0.68–1.56	0.88
rs3091244 (−286C→T/A)	0.86	0.67–1.10	0.23
rs1417938 (349T→A)	0.90	0.62–1.29	0.55
rs1800947 (1059G→C)	1.14	0.59–2.21	0.70
rs1130864 (1444C→T)	0.90	0.61–1.34	0.61
rs1205 (1846G→A)	1.15	0.78–1.68	0.49

* Adjusted for randomized treatment group, age, body mass index, history of hypertension, presence or absence of diabetes, smoking status, and cardiovascular disease status. Results are based on an additive model where genotypes were coded 0, 1, 2, and thus estimates presented are for subjects heterozygous for the risk allele at each locus. The OR for subjects homozygous for the risk allele is equal to the square of the OR for the heterozygotes.

These prospective data confirm the association between CFH Y402H and AMD. Incident cases are preferred over prevalent cases, because polymorphic genetic systems are often related to several conditions that could, at least in theory, alter the clinical course or survival of affected individuals and thus result in biased estimates of association. At least one prior report suggests that CFH Y402H is more strongly associated with advanced disease,[9] and consequently the magnitude of the association observed in any particular study is likely to be influenced by the distribution of case severity. In that regard, the present study included a majority of cases with early AMD, which may account for the more modest effect observed. In addition, the relatively small number of cases increases the chance that associations were underestimated. In contrast to *CFH*, we found no evidence that common genetic variants in *CRP* are associated with risk of AMD. Further prospective studies of larger groups of subjects are needed to understand the interrelations of these and other genes, as well as behavioral risk factors in the development of this important cause of blindness and visual impairment.

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