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Association of C-Reactive Protein Genetic Polymorphisms With Late Age-Related Macular Degeneration

Valentina Cipriani, PhD; Ruth E. Hogg, PhD; Reecha Sofat, PhD; Anthony T. Moore, FRCOphth; Andrew R. Webster, FRCOphth; John R. W. Yates, FRCP; Astrid E. Fletcher, PhD; for the European Eye (EUREYE) Study Group

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IMPORTANCE C-reactive protein (CRP) is a circulating inflammatory marker associated with late age-related macular degeneration (AMD). It remains uncertain whether the association between CRP concentrations and AMD is causal.

OBJECTIVE To assess whether *CRP* (OMIM 123260) single-nucleotide polymorphisms that influence circulating CRP concentrations are associated with late AMD.

DESIGN, SETTING, AND PARTICIPANTS Participants in 2 UK, hospital-based, case-control studies (Cambridge AMD study and Moorfields Eye Hospital AMD study) and 1 pan-European, cross-sectional, population-based study (the European Eye [EUREYE] Study) were recruited between November 6, 2000, and April 30, 2007. Participants underwent dilated stereo-digital fundus photography graded according to the International Classification of Age-related Maculopathy and Macular Degeneration. There were 1727 cases of late AMD (1151 neovascular, 384 geographic atrophy, and 192 mixed [neovascular AMD and geographic atrophy]) and 1153 controls. Early AMD cases (n = 574) were included only from the EUREYE Study. Data analysis was performed from August 1 to November 30, 2016. Four common single-nucleotide polymorphisms (rs1205, rs1130864, rs1800947, and rs3093077) were selected based on demonstrated influence on circulating CRP concentrations in the literature. In one study, genotyping of rs3093077 failed, and rs1800947 was typed in only 1 study.

MAIN OUTCOMES AND MEASURES A genetic multiplicative model was used for the association of single-nucleotide polymorphisms with late AMD adjusted for age and sex.

RESULTS Among the 1727 patients with late AMD, the mean (SD) age was 78.7 (7.4) years, and 668 (38.7%) were men. The mean (SD) age of the controls was 74.9 (7.0) years, and 510 (44.2%) were men. In the pooled results of all 3 studies, neither rs1205 (odds ratio [OR], 0.99; 95% CI, 0.86-1.14) nor rs1130864 (OR, 0.96; 95% CI, 0.83-1.11) was associated with late AMD. For geographic atrophy, rs1205 had an OR of 0.91 (95% CI, 0.74-1.13) and rs1130864 had an OR of 0.94 (95% CI, 0.76-1.16). For neovascular AMD, rs1205 had an OR of 1.01 (95% CI, 0.87-1.19) and rs1130864 had an OR of 0.99 (95% CI, 0.84-1.16). There was no association of rs3093077 and rs1800947 with late AMD or any late AMD phenotype. There were no significant findings for early AMD.

CONCLUSIONS AND RELEVANCE Our results do not support a causal association between CRP concentrations and AMD.

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The European Eye (EUREYE) Study Group members are listed at the end of this article.

Corresponding Author: Valentina Cipriani, PhD, UCL Institute of Ophthalmology, Department of Ocular Biology and Therapeutics, University College London, 11-43 Bath St, Wolfson Bldg, 3rd Floor, Room 3/5, London EC1V 9EL, England (v.cipriani@ucl.ac.uk).

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Inflammation has been recognized to play a key role in age-related macular degeneration (AMD),[1,2] strengthened by the discovery of multiple genetic risk loci involved in the complement pathway.[3-7] The early and intermediate stages of AMD are characterized by drusen, which are accumulations of lipids and proteins that form discrete deposits under the retinal pigment epithelium, and these accumulations can both initiate and reflect inflammatory reactions within the complement pathway.[1] Several studies have investigated the association between circulating inflammatory markers and the onset and progression of AMD,[8-12] both to understand the pathogenesis of AMD and to assess their potential as prognostic biomarkers. One such inflammatory marker is C-reactive protein (CRP), an acute phase reactant[13] that has been shown in 2 meta-analyses to be associated with late AMD.[14,15] C-reactive protein is involved in the initiation and modulation of the classical complement pathway, as well as the alternative complement pathway, through binding with complement factor H (*CFH* [OMIM 134370]). Therefore, it could be postulated that high concentrations of CRP play a causal role in the development of AMD by altering complement-related inflammatory responses to drusen.

Demonstration of a causal role of CRP in AMD is problematic, as is illustrated by the abundant work performed on CRP and coronary heart disease (CHD). Despite the numerous studies (including many prospective studies) consistently showing an increased risk of CHD with increasing CRP concentrations,[16] no studies have demonstrated an association between single-nucleotide polymorphisms (SNPs) in the *CRP* gene and the risk of CHD.[17,18] This approach, described as Mendelian randomization,[19] showed that, although certain *CRP* SNPs have been shown to influence the concentrations of circulating CRP,[20] the lack of an association between those *CRP* SNPs and CHD suggests that CRP is unlikely to have a causal influence on CHD.[17] A key point in the Mendelian randomization approach is that the causal role of a risk exposure for a specific disease is assessed by testing the association between genetic variants (that have been reliably shown to influence the exposure) and the disease of interest. The Mendelian randomization approach avoids bias due to reverse causation because genotypes are fixed at conception and, therefore, are not subject to confounding or modification by the onset of disease.[21]

Three previous studies have investigated the association between *CRP* genetic variants and AMD, but none have found an association.[22-24] The total number of late AMD cases in the 3 studies was 394, and each study may have lacked power. We investigated the association between AMD and *CRP* polymorphisms in 3 studies: the European Eye (EUREYE) Study, a cross-sectional, population-based epidemiologic study of AMD prevalence and risk factors,[25] and 2 hospital-based AMD case-control studies, the Cambridge AMD study[7] and the Moorfields Eye Hospital (MEH) AMD study.[26]

Key Points

Question Are variants in the C-reactive protein (*CRP*) gene that are known to influence circulating C-reactive protein concentrations associated with late age-related macular degeneration?

Findings This pooled analysis examined 4 *CRP* variants among 1727 patients with late age-related macular degeneration and 1153 controls from 2 hospital-based case-control studies and 1 cross-sectional population-based study. No statistical association was found between these *CRP* variants and any type of late age-related macular degeneration.

Meaning Circulating C-reactive protein concentrations are unlikely to be causally associated with age-related macular degeneration.

Methods

For all 3 studies, ethical approval was obtained from either national or local ethics committees (for the Cambridge AMD study, Anglia and Oxford Multicentre Research Ethics Committee; for the MEH AMD study, Moorfields and Whittington Local Research Ethics Committee; and for the EUREYE study, the local ethics committees of all 7 participating centers in 7 different countries). The study adhered to the tenets of the Declaration of Helsinki.[27] All participants provided written informed consent to undergo the clinical examination and epidemiologic data collection and to provide a blood sample for biochemical and genetic analyses.

The EUREYE Study

Participants were recruited between November 6, 2000, and November 15, 2002, by random sampling of the population 65 years of age or older in the following 7 centers: Bergen (Norway), Tallinn (Estonia), Belfast (United Kingdom), Paris-Creteil (France), Verona (Italy), Thessaloniki (Greece), and Alicante (Spain). The methods have been published in full elsewhere.[25] Trained field workers administered structured questionnaires including smoking status, diet, sunlight exposure, and self-reported medical history. The ophthalmic examination included dilated stereo-digital fundus photography. Stereo fundus images were graded at a single reading center (Erasmus University Rotterdam) according to the International Classification of Age-related Maculopathy and Macular Degeneration[28] and then categorized into 5 mutually exclusive grades. Grade 0 was defined as a macula free of drusen or pigmentary irregularities or with hard drusen (<63 µm) only. Early AMD was subdivided into grade 1, defined as soft distinct drusen (≥63 µm) or pigmentary abnormalities; grade 2, defined as soft indistinct drusen (≥125 µm), reticular drusen only, or soft distinct drusen (≥63 µm) with pigmentary abnormalities; and grade 3, defined as soft indistinct drusen (≥125 µm) or reticular drusen with pigmentary abnormalities. Grade 4 was defined as the presence of either neovascular AMD (nvAMD; the presence of any of the following: serous or hemorrhagic retinal or retinal pigment epithelial detachment, subretinal neovascular membrane, or periretinal fibrous scar) or geographic atrophy (GA; well-demarcated area of retinal pigment atrophy with visible choroidal vessels).

In the present analysis, for reason of costs, a random sample of controls (AMD stage 0) was frequency matched on age (within 1 year), sex, and center with patients with late AMD (nvAMD or GA) and patients with early AMD (stages 2 and 3). Nonfasting venous blood samples were collected at the ophthalmologic examination and separated within 4 hours of collection. Serum was stored at -20°C for up to 4 weeks before

frozen samples were transferred to a single laboratory (Queens University Belfast) for storage at -80°C.

The Cambridge AMD Study

The Cambridge AMD study is a case-control study of AMD with participants recruited from ophthalmic clinics in London, the southeast of England, and the northwest of England between November 1, 2001, and April 30, 2007.[7] All patients had at least 1 eye affected by nvAMD and/or GA. Patients were excluded if they had greater than 6 diopters of myopic refractive error or evidence of other inflammatory or retinovascular disease (such as retinal vessel occlusion, diabetic retinopathy, or chorioretinitis) that could contribute to the development of or confound the diagnosis of maculopathy. Almost all of the controls were spouses or partners of index patients, and the remainder were friends of patients. All participants described their race/ethnicity as white rather than other on a recruitment questionnaire. Participants were examined by an ophthalmologist and underwent color stereoscopic fundus photography of the macular region. Health, lifestyle, and smoking data were also collected from the participants. Images were graded at the Reading Centre, MEH, London, using the International Classification of Age-related Maculopathy and Macular Degeneration.[28] Blood samples were obtained at the time of interview; EDTA samples were obtained for DNA extraction, and lithium-heparin plasma samples stored at -80°C were later used for CRP measurements.

The MEH AMD Study

The MEH AMD study is a case-control study of AMD undertaken at MEH, London, between February 1, 2001, and January 31, 2004.[26] Only patients and controls of European origin were included in the study. Patients attending routine follow-up appointments and all new referrals from primary care clinics, casualty, and other centers with any type of AMD were enrolled to try to limit selection bias. Patients were excluded if they had retinochoroidal inflammatory disease, diabetic retinopathy, branch retinal vein or artery occlusion, or any other cause of visual loss other than amblyopia. Controls were recruited from spouses or friends of patients or from local residential homes for elderly persons within 8 km (5 miles) of the hospital. Each participant was interviewed specifically for the study, and a family history, smoking history, and other medical history were obtained. The ophthalmic examination included Snellen acuity, slitlamp examination, and biomicroscopic ophthalmoscopy. All participants underwent color stereoscopic fundus photography of the macular region, and the images were graded at the Reading Centre, MEH, London, using the International Classification of Age-related Maculopathy and Macular Degeneration.[28] Controls were excluded if drusen larger than 63 µm were evident. A sample of peripheral blood was obtained from each participant and stored at -20°C for DNA extraction and -80°C for analyte measurement.

Circulating CRP Concentrations

Circulating CRP concentrations were measured in the EUREYE and Cambridge AMD studies. In the EUREYE Study, the concentrations of serum CRP were assessed by a high-sensitivity, latex-enhanced turbidometric immunoassay (Wako Chemicals GmbH) using a Cobas Fara analyzer (Roche Diagnostics). In the Cambridge AMD study, the concentrations of plasma CRP were measured by a high-sensitivity, particle-enhanced immunonephelometric assay (Siemens CardioPhase hsCRP; Siemens Medical Solutions) using a Siemens Dade Behring BNII Nephelometer. Participants with CRP levels higher than 10 mg/L (to convert CRP to nanomoles per liter, multiply by 9.524), indicative of acute infection,[29] were not included in the analysis (90 from the EUREYE Study and 51 from the Cambridge AMD study). C-reactive protein concentrations were natural logarithmically transformed to ensure normality of the distribution and further Z transformed for investigation of the association of a 1-SD increase in log CRP with AMD.

Genotyping

We chose 4 common SNPs (rs1205, rs1130864, and rs1800947 in the 3′ UTR [untranslated region] and synonymous rs3093077 in exon 2) in the *CRP* gene previously reported in a meta-analysis on CRP and CHD using a Mendelian randomization approach.[17] These variants were selected as the most parsimonious set of tagging SNPs capturing maximum haplotype diversity and of demonstrated association with CRP concentrations among individuals of European descent.[21,30] The EUREYE Study samples were genotyped at LGC Genomics (Hoddesdon, England) using KASPar chemistry, a competitive allele-specific polymerase chain reaction SNP genotyping system using fluorescence resonance energy transfer quencher cassette oligos. Genotyping in the Cambridge AMD study was carried out using the ABI PRISM SNaPshot ddNTP Primer Extension Kit and a 3100 Genetic Analyzer (Applied Biosystems). Genotyping in the MEH AMD study was carried out using a Taqman (ABI) assay (Applied Biosystems). Departure from Hardy-Weinberg equilibrium was assessed for all SNPs in the control groups using a χ^2 goodness-of-fit test.

Statistical Analysis

Statistical analysis was performed from August 1 to November 30, 2016. We investigated the association of *CRP* genotype with late AMD and by late AMD phenotype (GA or nvAMD). We also report results for early AMD (available in the EUREYE Study only). All association analyses were conducted as 2-stage, fixed-effects meta-analysis of the available individual participant data from the 3 studies. Heterogeneity across study centers was assessed using the I^2 statistic. We calculated odds ratios (ORs) and their corresponding 95% CIs using logistic regression models adjusted for sex and age. Odds ratios were expressed as per 1 minor allele (multiplicative genetic model).

To confirm that, in our study population, the selected SNP genotypes influence circulating CRP concentrations, we examined the association of *CRP* genotype with CRP concentrations in controls via a Wald test on the genotype coded as 0, 1, and 2 according to the number of minor alleles using linear regression models adjusted for age and sex. We report geometric mean (SD) values for concentrations by genotype.

For comparison with previous studies, we analyzed the association of CRP concentrations with late AMD and late AMD phenotype using logistic regression models. These analyses

Table 1. Study Characteristics

Characteristic	EUREYE Study			Cambridge AMD Study		MEH AMD Study	
	Controls (n = 643)	Early AMD (n = 574)	Late AMD (n = 152)	Controls (n = 262)	Late AMD (n = 427)	Controls (n = 248)	Late AMD (n = 1148)
Age, mean (SD), y	74.7 (6.1)	74.6 (5.9)	79.5 (7.2)	75.7 (7.8)	80.4 (6.9)	74.8 (8.0)	77.9 (7.4)
Male, No. (%)	301 (46.8)	279 (48.6)	52 (34.2)	105 (40.1)	196 (45.9)	104 (41.9)	420 (36.6)
CRP, geometric mean (SD), mg/L	1.88 (1.21)	1.90 (1.28)	1.99 (1.35)	1.84 (1.72)	2.15 (1.92)	NA	NA
rs1205, No./Total No. (%)							
CC	273/612 (44.6)	246/560 (43.9)	58/146 (39.7)	119/256 (46.5)	200/419 (47.7)	101/231 (43.7)	478/1084 (44.1)
CT	267/612 (43.6)	240/560 (42.9)	75/146 (51.4)	113/256 (44.1)	183/419 (43.7)	107/231 (46.3)	507/1084 (46.8)
TT	72/612 (11.8)	74/560 (13.2)	13/146 (8.9)	24/256 (9.4)	36/419 (8.6)	23/231 (10.0)	99/1084 (9.1)
P value for HWE in controls	.36	NA	NA	.70	NA	.49	NA
MAF in controls	0.35	NA	NA	0.31	NA	0.33	NA
rs1130864, No./Total No. (%)							
CC	280/610 (45.9)	265/557 (47.6)	68/147 (46.3)	126/262 (48.1)	188/423 (44.4)	104/233 (44.6)	528/1084 (48.7)
CT	266/610 (43.6)	245/557 (44.0)	68/147 (46.3)	113/262 (43.1)	204/423 (48.2)	108/233 (46.4)	451/1084 (41.6)
TT	64/610 (10.5)	47/557 (8.4)	11/147 (7.4)	23/262 (8.8)	31/423 (7.3)	21/233 (9.0)	105/1084 (9.7)
P value for HWE in controls	.73	NA	NA	.74	NA	.35	NA
MAF in controls	0.35	NA	NA	0.30	NA	0.32	NA
rs3093077, No./Total No. (%)							
TT	NA[a]	NA[a]	NA[a]	232/262 (88.5)	380/425 (89.4)	211/233 (90.6)	960/1085 (88.5)
GT	NA[a]	NA[a]	NA[a]	30/262 (11.5)	45/425 (10.6)	21/233 (9.0)	119/1085 (11.0)
GG	NA[a]	NA[a]	NA[a]	0	0	1/233 (0.4)	6/1085 (0.5)
P value for HWE in controls	NA	NA	NA	.33	NA	.55	NA
MAF in controls	NA	NA	NA	0.06	NA	0.05	NA
rs1800947, No./Total No. (%)							
GG	519/585 (88.7)	471/539 (87.4)	113/136 (83.1)	NA	NA	NA	NA
CG	63/585 (10.8)	65/539 (12.1)	21/136 (15.4)	NA	NA	NA	NA
CC	3/585 (0.5)	3/539 (0.6)	2/136 (1.5)	NA	NA	NA	NA
P value for HWE in controls	.50	NA	NA	NA	NA	NA	NA
MAF in controls	0.07	NA	NA	NA	NA	NA	NA

Abbreviations: AMD, age-related macular degeneration; CRP, C-reactive protein; EUREYE, European Eye; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; MEH, Moorfields Eye Hospital; NA, not available.

SI conversion factor: To convert C-reactive protein to nanomoles per liter, multiply by 9.524.

[a] Genotyping for rs3093077 failed.

were adjusted for age and sex and, additionally, for a priori confounders including history of myocardial infarction or stroke, diabetes, and smoking. Odds ratios were expressed as per 1-SD change of log CRP concentration.

All statistical analyses were conducted using Stata software, version 13.1 (StataCorp), and ipdmetan and mvmeta commands were used for conducting meta-analyses of individual participant data. $P < .05$ was considered significant.

Results

There were 1727 cases of late AMD (1151 neovascular, 384 geographic atrophy, and 192 mixed [neovascular AMD and geographic atrophy]) and 1153 controls. Among the 1727 patients with late AMD, the mean (SD) age was 78.7 (7.4) years, and 668 (38.7%) were men. The mean (SD) age of the controls was 74.9 (7.0) years, and 510 (44.2%) were men. The characteristics of the participants and the genotype frequencies by study are shown in **Table 1**. Genotyping of rs3093077 failed in the EUREYE Study, and rs1800947 was not typed in the MEH AMD study and the Cambridge AMD study. In each study, no deviation from Hardy-Weinberg equilibrium was observed for any SNP, and control minimum allele frequencies (MAF) were similar both between studies and to those reported for the 1000 Genomes Project[31,32] Phase 3 European (EUR) samples: rs1205 MAF (T allele), 0.34; rs1130864 MAF (T allele), 0.33; rs3093077 MAF (G allele), 0.07; and rs1800947 MAF (C allele), 0.05. There were significant associations in the pooled analysis for the rs1205 T allele with lower CRP concentrations in controls and for the rs1130864 T allele with higher CRP concentrations in controls (**Table 2**). For the 2 rarer SNPs rs3093077 and rs1800947, there was no association with CRP concentrations in controls.

There was no evidence of any association of *CRP* SNPs rs1205, rs1130864, or rs3093077 with late AMD, or with type

of late AMD, either from the individual studies or in the meta-analysis, or for SNP rs1800947 in the EUREYE Study (**Table 3**).

A significant association was observed in the pooled age- and sex-adjusted analysis of CRP concentrations with late AMD (**Table 4**). Additional adjustment for smoking, myocardial infarction and stroke, and diabetes slightly reduced the ORs (from 1.19 to 1.16), and the *P* value changed from 0.03 to 0.07. Analyses of CRP concentrations by type of late AMD showed that GA had an OR of 1.35 (95% CI, 1.03-1.76; *P* = .03) and that nvAMD had an OR of 1.09 (95% CI, 0.91-1.29; *P* = .36).

Only the EUREYE Study had population-based data available on early AMD. There was no association of CRP concentrations with early AMD in age- and sex-adjusted or fully adjusted analyses (OR, 1.01; 95% CI, 0.82-1.25; *P* = .90) or of any *CRP* SNPs with early AMD (rs1205: OR, 1.08; 95% CI, 0.98-1.20; *P* = .13; rs1130864: OR, 0.89; 95% CI, 0.75-1.06; *P* = .20; and rs1800947: OR, 1.20; 95% CI, 0.89-1.63; *P* = .23).

Discussion

In this meta-analysis of 3 studies of AMD including 1727 patients with late AMD and 1153 controls, we found no evidence from analysis of *CRP* variants to support a causal association of CRP concentrations with late AMD, either nvAMD or GA. In agreement with many other studies,[17,18,33] we found that the T allele of rs1205 was associated with lower CRP concentrations in controls. However, there was no protective association between the T allele and either nvAMD or GA in any of the 3 studies. Similarly, the T allele of rs1130864 was associated with higher CRP concentrations in controls, but the T allele was not associated with higher odds of either late AMD phenotype.[17]

None of the 3 previous studies that investigated *CRP* polymorphisms and AMD found an association.[22-24] These studies had smaller sample sizes than our investigation and included two[23] or three[22,24] of the SNPs we investigated (rs1205, rs1130864, and rs1800947). In a case-control study (111 mixed early and late cases and 401 controls) nested within the prospective follow-up of the Physicians Health Study, AMD risk was not influenced by any of 7 *CRP* SNPs[24] or by any of 9 *CRP* SNPs in a sib-pair study of 112 cases of nvAMD.[22] The Rotterdam study (5861 participants, including 171 with late AMD) suggested that *CRP* haplotypes may modify the association of *CFH* Y402H genotype with late AMD, but the *CRP* haplotypes did not influence AMD risk directly; results were not presented for the 3 *CRP* SNPs individually.[23] In addition, a meta-analysis of 15 genome-wide association studies (GWASs)[34] and a recent large GWAS (16 144 patients and 17 832 controls)[35] of AMD did not show any genome-wide significant association signals at the *CRP* locus.

In our fully adjusted association analyses between CRP concentrations and AMD, we did not find a significant association with all cases of late AMD (*P* = .07; 1324 patients, including 489 with late AMD) and nvAMD (*P* = .36; 318 patients) or with early AMD (*P* = .90; 574 patients), which has been reported in other studies.[9,15,36] The association with AMD is not consistent, particularly in studies investigating early AMD.[11,37-40] In a large meta-analysis comparing low (<1 mg/L) vs high (>3 mg/L) CRP concentrations, Hong et al[14] reported an OR of 1.30 (95% CI, 1.03-1.65) in 3 studies that combined early and late AMD (4522 patients, including 1629 with AMD) and an OR of 2.19 (95% CI, 1.38-3.47) in 8 studies of late AMD (35 168 patients, including 1603 with AMD). Estimates for late AMD varied considerably according to method of AMD ascertainment, with much higher ORs reported for clinical diagnosis (3.8) compared with fundus photography (1.4), adjustment for diabetes (1.6) compared with no adjustment (2.5), and study design. Population-based cross-sectional or case-control studies had lower ORs than did clinic case-control studies or longitudinal studies. In a pooled analysis of prospective, nested case-control studies from 5 population-based US cohorts (647 patients, including 190 with nvAMD), CRP concentrations above 3 mg/L were associated with risk of all late AMD and nvAMD compared with concentrations less than 1 mg/L.[15]

Table 2. CRP Concentrations by Genotypes of *CRP* SNPs in Controls

CRP SNPs	EUREYE Study No.	EUREYE Study CRP, Geometric Mean (SD), mg/L	Cambridge AMD Study No.	Cambridge AMD Study CRP, Geometric Mean (SD), mg/L
rs1205				
CC	255	2.05 (1.36)	105	1.97 (1.83)
TC	249	1.79 (1.10)	96	1.82 (1.77)
TT	67	1.65 (1.01)	21	1.28 (1.25)
P value for trend of CRP concentration across genotypes[a]	NA	.003	NA	.13
Pooled *P* value for trend of CRP concentration across genotypes[b]	.001			
rs1130864				
CC	264	1.87 (1.24)	110	1.43 (1.42)
TC	248	1.84 (1.09)	94	2.32 (2.11)
TT	56	2.26 (1.65)	21	2.19 (1.29)
P value for trend of CRP concentration across genotypes[a]	NA	.19	NA	.002
Pooled *P* value for trend of CRP concentration across genotypes[b]	.01			
rs3093077[c]				
TT	NA	NA	200	1.82 (1.72)
GT	NA	NA	25	1.83 (1.91)
GG	NA	NA	0	NA
P value for trend of CRP concentration across genotypes[a]	NA	NA	NA	.83
rs1800947				
GG	486	1.87 (1.20)	NA	NA
GC	56	2.15 (1.30)	NA	NA
CC	3	1.11 (0.74)	NA	NA
P value for trend of CRP concentration across genotypes[a]	NA	.46	NA	NA

Abbreviations: AMD, age-related macular degeneration; CRP, C-reactive protein; EUREYE, European Eye; NA, not available; SNPs, single-nucleotide polymorphisms.

SI conversion factor: To convert C-reactive protein to nanomoles per liter, multiply by 9.524.

[a] From age- and sex-adjusted regression model of log CRP concentration in each study.

[b] From age- and sex-adjusted regression model of log CRP concentration in meta-analysis.

[c] Genotyping for rs3093077 failed.

Table 3. Association of *CRP* SNPs With Late AMD in the EUREYE Study, Cambridge AMD Study, and MEH AMD Study

	EUREYE Study[a]		Cambridge Study[a]		MEH Study[a]		Pooled[a]	
SNP	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Late AMD								
rs1205	1.06 (0.80-1.40)	.69	0.95 (0.73-1.22)	.66	0.98 (0.78-1.23)	.87	0.99 (0.86-1.14)	.88
rs1130864	0.86 (0.64-1.16)	.33	1.17 (0.90-1.52)	.24	0.89 (0.71-1.11)	.29	0.96 (0.83-1.11)	.60
rs3093077	NA	NA	0.92 (0.55-1.54)	.76	1.26 (0.80-1.98)	.32	1.10 (0.78-1.55)	.59
rs1800947	1.54 (0.95-2.50)	.08	NA	NA	NA	NA	NA	NA
Neovascular AMD								
rs1205	1.04 (0.75-1.44)	.81	0.99 (0.76-1.30)	.96	1.02 (0.80-1.29)	.88	1.01 (0.87-1.19)	.85
rs1130864	0.83 (0.58-1.17)	.28	1.29 (0.97-1.71)	.08	0.90 (0.71-1.13)	.35	0.99 (0.84-1.16)	.86
rs3093077	NA	NA	1.02 (0.59-1.77)	.95	1.25 (0.78-2.01)	.35	1.15 (0.80-1.64)	.45
rs1800947	1.68 (0.97-2.93)	.06	NA	NA	NA	NA	NA	NA
Geographic atrophy								
rs1205	1.11 (0.71-1.74)	.65	0.91 (0.60-1.38)	.65	0.85 (0.64-1.13)	.26	0.91 (0.74-1.13)	.40
rs1130864	0.98 (0.61-1.60)	.95	1.18 (0.79-1.76)	.42	0.83 (0.62-1.10)	.20	0.94 (0.76-1.16)	.59
rs3093077	NA	NA	0.73 (0.29-1.84)	.51	1.62 (0.94-2.77)	.08	1.31 (0.83-2.10)	.25
rs1800947	1.37 (0.62-3.04)	.43	NA	NA	NA	NA	NA	NA

Abbreviations: AMD, age-related macular degeneration; EUREYE, European Eye; MEH, Moorfields Eye Hospital; NA, not available; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] Sex- and age-adjusted OR and *P* value for a genetic multiplicative model.

Table 4. Association of C-Reactive Protein Concentration With Late AMD

	Late AMD			Neovascular AMD			Geographic Atrophy		
Value	EUREYE Study	Cambridge AMD Study	Pooled (n = 1324)	EUREYE Study	Cambridge AMD Study	Pooled (n = 1153)	EUREYE Study	Cambridge AMD Study	Pooled (n = 946)
OR (95% CI)[a,b]	1.17 (0.89-1.56)	1.20 (1.00-1.45)	1.19 (1.02-1.39)	1.09 (0.79-1.51)	1.13 (0.93-1.38)	1.12 (0.95-1.33)	1.33 (0.84-2.09)	1.48 (1.09-2.02)	1.43 (1.11-1.84)
P value	.26	.05	.03	.59	.23	.19	.22	.01	.006
OR (95% CI)[c]	1.10 (0.82-1.47)	1.18 (0.98-1.43)	1.16 (0.99-1.36)	1.00 (0.71-1.41)	1.12 (0.91-1.37)	1.09 (0.91-1.29)	1.26 (0.79-2.03)	1.39 (1.01-1.92)	1.35 (1.03-1.76)
P value	.54	.08	.07	.99	.29	.36	.33	.05	.03

Abbreviations: AMD, age-related macular degeneration; EUREYE, European Eye; OR, odds ratio.

[a] Odds ratio per 1-SD change in log C-reactive protein concentration.

[b] Adjusted for age and sex.

[c] Adjusted for age, sex, diabetes, cardiovascular disease, and smoking status.

Limitations

Although our study was larger than the 3 previous studies that investigated the association between *CRP* variants and AMD, it does have several limitations, especially with respect to the association between CRP concentrations and AMD. In the EUREYE and Cambridge studies, the blood samples used for measuring CRP concentrations were collected at the same time as ascertainment of AMD, and therefore we cannot exclude reverse causation (eg, an inflammatory response) due to risk factors for AMD, such as atherosclerosis and cardiovascular disease. We only had 1 measure of CRP, which may not represent longer-term usual concentrations. In addition, circulating CRP concentrations may not adequately reflect the CRP concentration in the relevant tissues. The monomeric form of CRP has been shown to be responsible for the upregulation of proinflammatory cytokines interleukin 8 and chemokine ligand 2 in retinal pigment epithelial cells, and patients with AMD carrying the risk variants of *CFH* Y204H showed impaired binding of monomeric CRP.[41]

Genetic variants in *CRP* are not subject to reverse causation[19,21] and, therefore, provide stronger evidence on the possible association of CRP concentrations with AMD. We investigated only a small number of *CRP* SNPs. Nevertheless, we selected SNPs expected to show a causal association with AMD because these SNPs influence CRP concentrations.[17,20,21,30] Variants associated with CRP concentrations in other loci have been identified in large GWASs and replication studies.[18,42] These variants are found in genes primarily involved in lipid, metabolic, or immune pathways and include *IL6R* (OMIM 147880), *HNF1A* (OMIM 142410), and *APOCI* (OMIM 107710). In a GWAS of CHD, the *APOC1* variant (rs4420638) was associated with increased risk of CHD but in an opposite direction to that anticipated from its association with CRP and consistent with its association with low-density lipoprotein and high-density lipoprotein cholesterol and triglycerides.[18] This variant has also been identified at genome-wide significance to increase the risk of AMD.[34] *APOC1* is part of the apolipoprotein gene cluster that plays a major role in lipid transport and metabolism and includes the well-established *APOE* (OMIM 107741) AMD risk variant (rs429358).[43] Linkage disequilibrium (r^2 = 0.7)[44] is observed between rs4420638 and rs429358 in the EUR populations from Phase 3 of the 1000 Genomes Project.[31,32]

Conclusions

From the analogy with CHD[17] and the identification of alternative genetic loci for CRP concentrations already discussed, along with the lack of association of variants in the *CRP* locus with AMD shown in our meta-analysis corroborating results from previous studies, we conclude that CRP concentrations are unlikely to be causally associated with AMD.

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Author Affiliations: Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, University College London, London, England (Cipriani, Moore, Webster, Yates); Moorfields Eye Hospital, London, England (Cipriani, Moore, Webster); University College London Genetics Institute, London, England (Cipriani); Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland (Hogg); Centre for Clinical Pharmacology, Division of Medicine, University College London, London, England (Sofat); Ophthalmology Department, School of Medicine, University of California, San Francisco (Moore); Department of Medical Genetics, University of Cambridge, Cambridge, England (Yates); Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, England (Fletcher).

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Study concept and design: Cipriani, Sofat, Fletcher.
Acquisition, analysis, or interpretation of data: All authors.
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Critical revision of the manuscript for important intellectual content: Hogg, Sofat, Moore, Webster, Yates, Fletcher.
Statistical analysis: Cipriani.
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Group Information: The EUREYE Study group members include Astrid E. Fletcher, PhD (London School of Hygiene and Tropical Medicine, London, England); Graham Bentham, MA (University of East Anglia, Norwich, England); Usha Chakravarthy, MD (Queen's University Belfast, Belfast, United Kingdom); Paulus TVM de Jong, MD (Netherlands Institute for Ophthalmic Research, Amsterdam, Netherlands); Mati Rahu, PhD (National Institute for Health Development, Tallinn, Estonia); John Seland, MD (University of Bergen, Bergen, Norway); Gisele Soubrane, MD (Universitaire de Créteil, Paris, France); Laura Tomazzoli, MD (Università degli studi di Verona, Verona, Italy); Fotis Topouzis, MD (Aristotle University of Thessaloniki, Thessaloniki, Greece); Jesus Vioque, MD (Universidad Miguel Hernandez, Alicante, Spain); and Ian Young, MD (Queen's University Belfast, Belfast, United Kingdom).

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Invited Commentary

Association Between C-Reactive Protein and Age-Related Macular Degeneration

Les Liaisons Dangereuses

Tiarnán D. Keenan, MD, PhD; Emily Y. Chew, MD

Triangular relationships might make life interesting, but they can be hard to untangle. In this issue of *JAMA Ophthalmology*, Cipriani et al[1] have conducted an elegant study examining the triangular association between circulating concentrations of C-reactive protein (CRP), *CRP* (OMIM 123260) genetic variants, and risk of late age-related macular degeneration (AMD) (choroidal neovascularization or central geographic atrophy). Their findings have important implications for our understanding of the pathophysiology of AMD, particularly the distinction between systemic and local inflammation.

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The authors pooled data from 3 European studies to test potential associations in this triangular affair. Two *CRP* genetic variants did share some association with circulating CRP concentrations (in control participants). However, no genetic variant was significantly associated with the risk of AMD; previous observers[2] have also searched for this association in vain. In any case, as the authors helpfully pointed out by analogy with cardiovascular research, there is a big difference between association and causation.

In the study by Cipriani et al,[1] circulating CRP concentrations were weakly associated with geographic atrophy, along partly similar lines to those of findings for late AMD in some previous studies. It is important to remember, however, that circulating