Original Investigation | EPIDEMIOLOGY

Markers of Inflammation, Oxidative Stress, and Endothelial Dysfunction and the 20-Year Cumulative Incidence of Early Age-Related Macular Degeneration
The Beaver Dam Eye Study

Ronald Klein, MD, MPH; Chelsea E. Myers, MStat; Karen J. Cruickshanks, PhD; Ronald E. Gangnon, PhD; Lorraine G. Danforth, BS; Theru A. Sivakumaran, PhD; Sudha K. Iyengar, PhD; Michael Y. Tsai, PhD; Barbara E. K. Klein, MD, MPH

IMPORTANCE Modifying levels of factors associated with age-related macular degeneration (AMD) may decrease the risk for visual impairment in older persons.

OBJECTIVE To examine the relationships of markers of inflammation, oxidative stress, and endothelial dysfunction to the 20-year cumulative incidence of early AMD.

DESIGN, SETTING, AND PARTICIPANTS This longitudinal population-based cohort study involved a random sample of 975 persons in the Beaver Dam Eye Study without signs of AMD who participated in the baseline examination in 1988-1990 and up to 4 follow-up examinations in 1993-1995, 1998-2000, 2003-2005, and 2008-2010.

EXPOSURES Serum markers of inflammation (high-sensitivity C-reactive protein, tumor necrosis factor–α receptor 2, interleukin-6, and white blood cell count), oxidative stress (8-isoprostane and total carbonyl content), and endothelial dysfunction (soluble vascular cell adhesion molecule–1 and soluble intercellular adhesion molecule–1) were measured. Interactions with complement factor H (rs1061170), age-related maculopathy susceptibility 2 (rs10490924), complement component 3 (rs2230199), and complement component 2/complement factor B (rs4151667) were examined using multiplicative models. Age-related macular degeneration was assessed from fundus photographs.

MAIN OUTCOMES AND MEASURES Early AMD defined by the presence of any size drusen and the presence of pigmentary abnormalities or by the presence of large-sized drusen (≥125-μm diameter) in the absence of late AMD.

RESULTS The 20-year cumulative incidence of early AMD was 23.0%. Adjusting for age, sex, and other risk factors, high-sensitivity C-reactive protein (odds ratio comparing fourth with first quartile, 2.18; $P = .005$), tumor necrosis factor–α receptor 2 (odds ratio, 1.78; $P = .04$), and interleukin-6 (odds ratio, 1.78; $P = .03$) were associated with the incidence of early AMD. Increased incidence of early AMD was associated with soluble vascular cell adhesion molecule–1 (odds ratio per SD on the logarithmic scale, 1.21; $P = .04$).

CONCLUSIONS AND RELEVANCE We found modest evidence of relationships of serum high-sensitivity C-reactive protein, tumor necrosis factor–α receptor 2, interleukin-6, and soluble vascular cell adhesion molecule–1 to the 20-year cumulative incidence of early AMD independent of age, smoking status, and other factors. It is not known whether these associations represent a cause and effect relationship or whether other unknown confounders accounted for the findings. Even if inflammatory processes are a cause of early AMD, it is not known whether interventions that reduce systemic inflammatory processes will reduce the incidence of early AMD.

JAMA Ophthalmol. 2014;132(4):446-455. doi:10.1001/jamaophthalmol.2013.7671
Published online January 30, 2014.

Supplemental content at jamaophthalmology.com

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

Corresponding Author: Ronald Klein, MD, MPH, University of Wisconsin–Madison, School of Medicine and Public Health, Department of Ophthalmology and Visual Sciences, 610 N Walnut St, 4th Fl, WARF, Madison, WI 53726-2336 (kleinr@epi.ophth.wisc.edu).

Section Editor: Leslie Hyman, PhD.

Age-related macular degeneration (AMD), the most common cause of severe loss of vision in older persons of European ancestry, is a multifactorial disease with strong evidence of genetic determinants.[1-6] Age, smoking status, physical activity, and obesity have been found in most studies to be related to the incidence of AMD.[6] Inflammation, oxidative stress, and endothelial dysfunction are also among the many host and environmental influences that have been hypothesized to affect the incidence and progression of AMD.[7-25] There is a strong biological rationale supporting a role of inflammation, oxidative stress, and, to a lesser extent, endothelial dysfunction in the development and progression of AMD. There is accumulating evidence of a relationship of high-sensitivity C-reactive protein (hsCRP) to late AMD but less consistent evidence of a similar relationship to the incidence of early AMD.[26-36] To our knowledge, few epidemiological studies have examined the relationships of other systemic markers of inflammation (eg, tumor necrosis factor-α receptor 2 [TNF-αR2]),[32-34] oxidative stress,[37] and endothelial dysfunction[32,34,38] to the incidence of AMD.

In an earlier prospective substudy in the Beaver Dam Eye Study (BDES) cohort, we found no relationships of markers of systemic inflammation and endothelial dysfunction to the 10-year cumulative incidence of early AMD.[33] Since that report, we have genotyped AMD candidate gene single-nucleotide polymorphisms (SNPs) including complement factor H (*CFH*, rs1061170), age-related maculopathy susceptibility 2 (*ARMS2*, rs10490924), complement component 2/complement factor B (*C2/CFB*, rs4151667), and complement component 3 (*C3*, rs2230199) and remeasured the same markers as well as systemic markers of oxidative stress present in a random sample of the BDES cohort. We hypothesized that elevated levels of markers of systemic inflammation in the presence of 1 or 2 variant alleles for *CFH* rs1061170, *ARMS2* rs10490924, *C2/CFB* rs4151667, and *C3* rs2230199 and higher levels of markers of oxidative stress and endothelial dysfunction would be associated with a greater risk for developing early AMD.

Methods

Population

Methods used to identify and descriptions of the population have appeared in previous reports.[39-43] Of the 5924 eligible persons identified by a private census, 4926 (83%) persons aged 43 to 86 years participated in the baseline examination in 1988-1990. Ninety-nine percent of the population was white. The cohort was reexamined at 5- (n = 3722), 10- (n = 2962), 15- (n = 2375), and 20-year (n = 1913) follow-up examinations.[40-43] There was greater than 80% participation among survivors at each examination.

All data were collected with institutional review board approval from the University of Wisconsin-Madison in conformity with all federal and state laws; the work was compliant with the Health Insurance Portability and Accountability Act, and the study adhered to the tenets of the Declaration of Helsinki. Informed written consent was obtained from each participant before every examination. Comparisons between participants and nonparticipants at each examination have appeared elsewhere.[39-43] In general, those who participated in the follow-up were more likely to be younger than nonparticipants who were alive or those who died and, while adjusting for age, were less likely to have AMD.

Procedures

A standardized interview and examination were administered at each visit. Information on demographic characteristics; medication use, including history of use of lipid-lowering drugs by type and use of steroidal and nonsteroidal anti-inflammatory drugs; and history of smoking and physical activity was obtained by questionnaire. Body weight and height were measured. Similar procedures were followed at baseline and follow-up examinations.[44]

Causal blood specimens were obtained at the baseline examination. An aliquot of blood was used immediately to determine the white blood cell (WBC) count. Remaining serum was stored for up to 17 years until being shipped on dry ice to the University of Minnesota laboratory for measurement of markers of inflammation (hsCRP, interleukin-6 [IL-6], and TNF-αR2), oxidative stress (8-isoprostane [8-ISO], an indicator of lipid oxidation, and total carbonyl content [TCC], an indicator of the amount of protein that has been oxidized by highly reactive free radicals), and endothelial dysfunction (soluble vascular cell adhesion molecule-1 [sVCAM-1] and soluble intercellular adhesion molecule-1 [sICAM-1]). The eAppendix (Supplement) describes the procedures to measure these markers and their interassay coefficients of variation as well as measurements of candidate gene SNPs.[45]

Fundus Photography and Grading

Stereoscopic 30° color film fundus photographs centered on the macula (Diabetic Retinopathy Study standard field 2) were taken of each eye.[44,46,47] Gradings were performed for the pair of photographs of each macula at each examination using the Wisconsin Age-Related Maculopathy Grading System.[46-51] Graders were masked to any information regarding the participant and the fellow eye.

Definitions

The severity of AMD was determined using the 5-step Three Continent AMD Consortium Severity Scale.[52] Individuals were considered not to have AMD if both eyes had either hard drusen or small soft drusen (<125 μm in diameter) only, regardless of the area of involvement and no pigmentary abnormalities (defined as increased retinal pigment or retinal pigment epithelial [RPE] depigmentation present) or no definite drusen with any pigmentary abnormality. Early AMD was defined by the presence of any sized drusen and the presence of any pigmentary abnormality or by the presence of large-sized drusen (≥125 μm in diameter), regardless of the area of involvement, in the absence of late AMD defined by the presence of pure geographic atrophy or exudative macular degeneration. When 1 eye was ungradable, it was assumed to have the same AMD severity as the fellow eye.

Persons at risk for developing early AMD were those without early AMD in either eye at baseline. The incidence of early

AMD was defined by developing signs of early AMD in at least 1 eye when both eyes had no AMD at the baseline examination. Incidence was determined for signs of early AMD (eg, large drusen size ≥125 µm, drusen type [soft indistinct/reticular], and pigmentary abnormalities [increased retinal pigment and RPE depigmentation]). Owing to limited power, we did not examine the relationship of the markers and risk for developing late AMD.

All covariates were measured at baseline. Age was categorized into 4 groups: 43 to 54 years, 55 to 64 years, 65 to 74 years, and 75 or more years. Body mass index (BMI) was calculated as a participant's weight in kilograms divided by their height in meters squared. Obesity was defined as a BMI of 30 or greater. Current smokers were identified as persons having smoked 100 or more cigarettes in their lifetime and smoking at the time of the examination. Participants were considered physically active if they engaged in physical activity long enough to work up a sweat at least once per week. The use of statin drugs, steroidal anti-inflammatory drugs, and nonsteroidal anti-inflammatory drugs was determined from self-report.

Statistical Analysis

All analyses were performed with SAS version 9.2 (SAS Institute). Cumulative incidence was estimated by the product-limit method,[53] accounting for the competing risk for death.[54] Discrete logistic hazard regression[55] was used to estimate odds ratios (ORs) for associations between each marker of inflammation, oxidative stress, and endothelial dysfunction with incidence of early AMD, incidence of large drusen greater than or equal to 125 µm in diameter, incidence of soft indistinct or reticular drusen, and incidence of pigmentary abnormalities. Each marker was examined using a natural logarithmic transformation and categorized into quartiles. *P* values are reported per SD increase on the logarithmic scale, for each higher quartile compared with the first quartile and for a trend per increasing quartile. Models first adjusted only for age and sex. Then models additionally adjusted for smoking status, physical activity, BMI, statin use, and anti-inflammatory medication use. Odds ratios were estimated for associations of having 1 and 2 vs 0 risk alleles of *CFH* and *ARMS2* and having 1 or 2 vs 0 risk alleles for *C3* and *C2/CFB* with the incidence of early AMD adjusting for the same factors. *P* values were estimated for the relationship of age (older 2 vs younger 2 age groups), sex, obesity, current smoking status, and physical activity to each marker using the Mann-Whitney *U* test. To test for interactions, we first modeled a multiplicative interaction between each inflammatory, oxidative stress, and endothelial dysfunction marker and having 0, 1, or 2 risk alleles for *CFH* and *ARMS2* and having 0, 1, or 2 risk alleles for *C3* and *C2/CFB*; we then examined each relationship by stratifying by genotype for each SNP.

Change in area under the receiver operating characteristic curve was used to measure improvement in prediction when a marker (eg, hsCRP, modeled as trend per SD on the logarithmic scale) was added to a model based on traditional AMD risk factors and to a model based on traditional risk factors plus candidate SNPs using the method described by DeLong and colleagues.[56]

Results

Of the 4926 BDES participants at baseline, 1793 were included as part of a random sample in a substudy of chronic kidney disease. Age, sex, BMI, history of smoking, history of sedentary lifestyle, history of use of nonsteroidal anti-inflammatory drugs, presence of early and late AMD, and the distributions of the AMD candidate genotypes did not differ between those included in the random sample and those excluded, except for the *CFH* variant allele (59% in those included vs 62% in those excluded; *P* = .03; eTable 1 in Supplement). To be included in analyses, a participant from the random sample must have had measures of markers of inflammation (hsCRP, IL-6, TNF-αR2, and WBC count), oxidative stress (8-ISO and TCC), and endothelial dysfunction (sVCAM-1 and sICAM-1), relevant genetic data, and no AMD at baseline as assessed from 30° stereoscopic color fundus photographs. Each participant also must have had at least 1 follow-up visit with photographs where at least 1 eye was gradable for AMD. Characteristics of the 975 persons who met these criteria and were included in analyses and those excluded are described in **Table 1**.

Associations of Markers With the 20-Year Incidence of Early AMD

One hundred ninety-eight of the 975 individuals developed early AMD. The 20-year cumulative incidence adjusting for the competing risk for death for early AMD was 23.0% (95% CI, 20.2-25.8). Log-transformed serum hsCRP (*P* = .004), IL-6 (*P* = .02), and sVCAM-1 (*P* = .04) were associated with the 20-year cumulative incidence of AMD while adjusting for age, sex, and other factors (**Table 2**). There were trends for increasing quartile of hsCRP (*P* for test of trend = .01) and IL-6 (*P* for test of trend = .04) and higher 20-year cumulative incidence of early AMD. Compared with those in the lowest quartile, those in the highest quartile for hsCRP (*P* = .005), TNF-αR2 (*P* = .04), and IL-6 (*P* = .03) had greater odds of developing early AMD (Table 2). When all 3 of these markers plus sVCAM-1 were included in the same model, only hsCRP (*P* = .04) and sVCAM-1 (*P* = .04) remained associated with the incidence of early AMD. There were no other relationships of other markers to the 20-year cumulative incidence of early AMD (Table 2). Relationships for hsCRP and WBC count were similar when analyses were expanded to include all individuals in the population (data not shown). The relationships of the markers of inflammation, oxidative stress, and endothelial dysfunction to the incidence of drusen type and size and pigmentary abnormalities were similar to that of early AMD (eTable 2 in Supplement).

We examined the relationship of serum TNF-αR2 to the incidence of early AMD by sex and, in women, by menopausal status. The TNF-αR2 relationship was similar in women (OR per 1 SD increase on logarithmic scale, 1.22; *P* = .08) and men (OR, 1.16; *P* = .33), and it was stronger in women who had gone through menopause (OR, 1.45; *P* = .01) compared with women who had not (OR, 1.07; *P* = .91), while adjusting for age, BMI, smoking status, physical activity levels, and use of statins and anti-inflammatory medications.

Table 1. Characteristics of Participants Included and Excluded From Analysis

Measure	Included		Excluded		*P* Value[a]
	No.	Mean (SD)	No.	Mean (SD)	
Age, y	975	58.1 (9.8)	3951	63.0 (11.3)	<.001
Men, %	421	43.2	1743	44.1	.60
BMI	972	28.8 (5.6)	3909	28.7 (5.4)	.92
Smoking status, %					
Former	334	34.3	1413	35.8	.63
Current	205	21.0	765	19.4	.37
Sedentary lifestyle, %	707	72.5	3068	77.7	.001
Using NSAIDs, %	303	31.1	1340	33.9	.09
CFH genotype, %					
C/T	408	44.4	1701	48.2	.009
C/C	115	12.5	486	13.8	.06
ARMS2 genotype, %					
G/T	346	36.4	1245	34.5	.45
T/T	35	3.7	193	5.3	.05
C2/CFB rs4151667 genotype, %					
T/A	42	6.4	223	9.9	.006
A/A	0	0.0	8	0.4	.97
C3 rs2230199 genotype, %					
C/G	217	33.0	699	30.9	.33
G/G	25	3.8	94	4.2	.79
Median hsCRP, mg/L	956	1.9			
Median TNF-αR2, pg/mL	950	2295.2			
Median IL-6, pg/mL	944	2.1			
Median WBC count, 1000/μL	957	7.0			
Median 8-ISO, pg/mL	961	120.7			
Median TCC, nmol/mg	971	0.1			
Median sVCAM-1, ng/mL	973	758.8			
Median sICAM-1, ng/mL	970	277.9			

Abbreviations: A, adenine; *ARMS2*, age-related maculopathy susceptibility 2; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); C, cytosine; *CFH*, complement factor H; *C2/CFB*, complement component 2/complement factor B; *C3*, complement component 3; G, guanine; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; 8-ISO, 8-isoprostane; NSAID, nonsteroidal anti-inflammatory drug; sICAM-1, serum intercellular adhesion molecule–1; sVCAM-1, serum vascular cell adhesion molecule–1; T, thymine; TCC, total carbonyl content; TNF-αR2, tumor necrosis factor–α receptor 2; WBC, white blood cell.

SI conversion factors: To convert hsCRP to nmol/L, multiply by 9.524; and WBC count to $\times 10^9$/L, multiply by 0.001.

[a] Adjusted for age and sex.

Associations of Candidate Gene SNPs With the 20-Year Incidence of Early AMD and Interactions With the Markers

Both *CFH* ($P = .003$) and *ARMS2* ($P = .006$) with 2 risk alleles were associated with the 20-year incidence of early AMD. However, neither *C3* nor *C2/CFB* were related to the 20-year incidence of early AMD (eTable 3 in Supplement). The relationships of each marker per SD on the logarithmic scale, stratified by having 0, 1, and 2 risk alleles for *CFH* and *ARMS2*, to the incidence of early AMD after adjustment for age, sex, smoking status, and other factors at baseline are presented in the **Figure**. There was a borderline multiplicative interaction of hsCRP ($P = .08$) and WBC count ($P = .08$) and *CFH*, and an inverse interaction of TNF-αR2 ($P < .001$) and sICAM-1 ($P = .04$) and *ARMS2* and the incidence of early AMD. There were no interactions between *C3* or *C2/CFB* and any of the markers (data not shown).

Risk Assessment

The models including smoking status, physical activity, BMI, age, and sex discriminated poorly in predicting the incidence of early AMD (**Table 3**). The largest increase and incremental gain in the area under the receiver operating characteristic curve occurred after including hsCRP in the model that included traditional risk factors for incidence of early AMD; however, it was not statistically significant ($P = .42$, Table 3).

Discussion

In the BDES, higher levels of serum hsCRP, TNF-αR2, IL-6, and sVCAM-1 were modestly associated with the 20-year cumulative incidence of early AMD independent of age, sex, smoking status, physical activity, obesity status, and history of use of statins and anti-inflammatory drugs.

Most studies have shown a consistent relationship between serum hsCRP and late AMD.[26-28,31,33-36] There is less consistency in studies that have examined the relationship of hsCRP to the long-term incidence of early AMD; 5 studies did not find a relationship[26,28,30,31,34] and 2 did.[27,36] Our findings are consistent with those from a recent meta-analysis of 5 large studies that showed that participants with high hsCRP levels (>3 mg/L; to convert to nanomoles per liter, multiply by 9.524) had an increased risk for incident early AMD (OR, 1.49; 95% CI, 1.06-2.08) compared with participants with low hsCRP levels (<1 mg/L).[36] When similar analyses were performed in the BDES cohort, while adjusting for age, sex,

Table 2. Relationship of Inflammatory, Oxidative Stress, and Endothelial Dysfunction Markers to the 20-Year Cumulative Incidence of Early AMD

Marker	No. at Risk	No. of Events	Model 1[a]		Model 2[b]	
			OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
hsCRP, mg/L						
Per SD on log scale	875	178	1.27 (1.08-1.50)	.003	1.29 (1.08-1.55)	.004
≤1.0	237	33	1.00		1.00	
>1.0-2.0	226	54	1.77 (1.12-2.80)	.02	1.94 (1.19-3.14)	.007
>2.0-4.5	247	50	1.55 (0.98-2.46)	.06	1.76 (1.07-2.87)	.03
>4.5	165	41	2.14 (1.31-3.48)	.002	2.18 (1.27-3.74)	.005
Trend over quartiles	875	178	1.22 (1.06-1.42)	.007	1.23 (1.05-1.45)	.01
TNF-αR2, pg/mL						
Per SD on log scale	886	179	1.18 (1.00-1.40)	.06	1.19 (1.00-1.42)	.06
≤2000	252	36	1.00		1.00	
>2000-2500	320	71	1.55 (1.02-2.36)	.04	1.56 (1.01-2.42)	.05
>2500-3000	176	37	1.48 (0.91-2.41)	.12	1.44 (0.87-2.39)	.16
>3000	138	35	1.76 (1.04-2.99)	.04	1.78 (1.03-3.08)	.04
Trend over quartiles	886	179	1.17 (1.00-1.38)	.05	1.17 (0.99-1.38)	.07
IL-6, pg/mL						
Per SD on log scale	880	176	1.22 (1.05-1.42)	.009	1.22 (1.03-1.44)	.02
≤1.6	274	43	1.00		1.00	
>1.6-2.4	244	52	1.50 (0.98-2.29)	.06	1.58 (1.00-2.50)	.05
>2.4-3.7	192	42	1.45 (0.92-2.29)	.11	1.57 (0.96-2.56)	.07
>3.7	170	39	1.69 (1.06-2.69)	.03	1.78 (1.05-3.02)	.03
Trend over quartiles	880	176	1.17 (1.01-1.35)	.03	1.19 (1.01-1.39)	.04
WBC count, 1000/μL						
Per SD on log scale	892	181	1.23 (1.05-1.45)	.01	1.13 (0.95-1.35)	.16
≤6.0	241	40	1.00		1.00	
>6.0-7.2	245	58	1.49 (0.97-2.28)	.07	1.45 (0.93-2.25)	.10
>7.2-8.5	200	39	1.26 (0.79-2.01)	.34	1.13 (0.69-1.84)	.63
>8.5	206	44	1.75 (1.11-2.75)	.02	1.41 (0.86-2.31)	.17
Trend over quartiles	892	181	1.16 (1.01-1.33)	.04	1.08 (0.92-1.26)	.34
8-ISO, pg/mL						
Per SD on log scale	879	175	0.95 (0.81-1.12)	.55	0.92 (0.78-1.08)	.30
≤97	232	43	1.00		1.00	
>97-124	229	54	1.37 (0.89-2.10)	.15	1.43 (0.92-2.21)	.11
>124-164	201	46	1.41 (0.90-2.19)	.13	1.53 (0.97-2.42)	.07
>164	217	32	0.79 (0.49-1.27)	.32	0.71 (0.43-1.17)	.18
Trend over quartiles	879	175	0.94 (0.82-1.08)	.41	0.92 (0.80-1.07)	.28
TCC, nmol/mg						
Per SD on log scale	888	180	0.98 (0.83-1.15)	.80	0.97 (0.82-1.15)	.75
≤0.1	255	51	1.00		1.00	
>0.1-0.14	182	35	0.94 (0.60-1.47)	.78	0.87 (0.54-1.39)	.56
>0.14-0.2	213	54	1.26 (0.84-1.90)	.27	1.19 (0.78-1.82)	.42
>0.2	238	40	0.88 (0.57-1.36)	.56	0.88 (0.56-1.38)	.57
Trend over quartiles	888	180	0.99 (0.87-1.14)	.92	0.99 (0.86-1.14)	.92
sVCAM-1, ng/mL						
Per SD on the log scale	890	180	1.17 (0.99-1.39)	.06	1.21 (1.01-1.44)	.04
≤660	256	44	1.00		1.00	
>660-790	258	50	1.14 (0.74-1.74)	.55	1.19 (0.76-1.85)	.45
>790-950	211	42	1.13 (0.72-1.78)	.58	1.14 (0.72-1.81)	.58
>950	165	44	1.43 (0.90-2.27)	.13	1.58 (0.98-2.56)	.06
Trend over quartiles	890	180	1.11 (0.96-1.29)	.16	1.14 (0.98-1.33)	.09
sICAM-1, ng/mL						

(continued)

Table 2. Relationship of Inflammatory, Oxidative Stress, and Endothelial Dysfunction Markers to the 20-Year Cumulative Incidence of Early AMD (continued)

Marker	No. at Risk	No. of Events	Model 1[a]		Model 2[b]	
			OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Per SD on the log scale	888	180	1.04 (0.89-1.23)	.61	0.98 (0.82-1.17)	.81
≤240	248	45	1.00		1.00	
>240-280	207	48	1.36 (0.88-2.09)	.17	1.49 (0.95-2.34)	.08
>280-330	212	43	1.07 (0.69-1.66)	.77	1.08 (0.68-1.72	.74
>330	221	44	1.26 (0.81-1.96)	.30	1.10 (0.68-1.78)	.70
Trend over quartiles	888	180	1.05 (0.91-1.20)	.50	1.00 (0.86-1.16)	.99

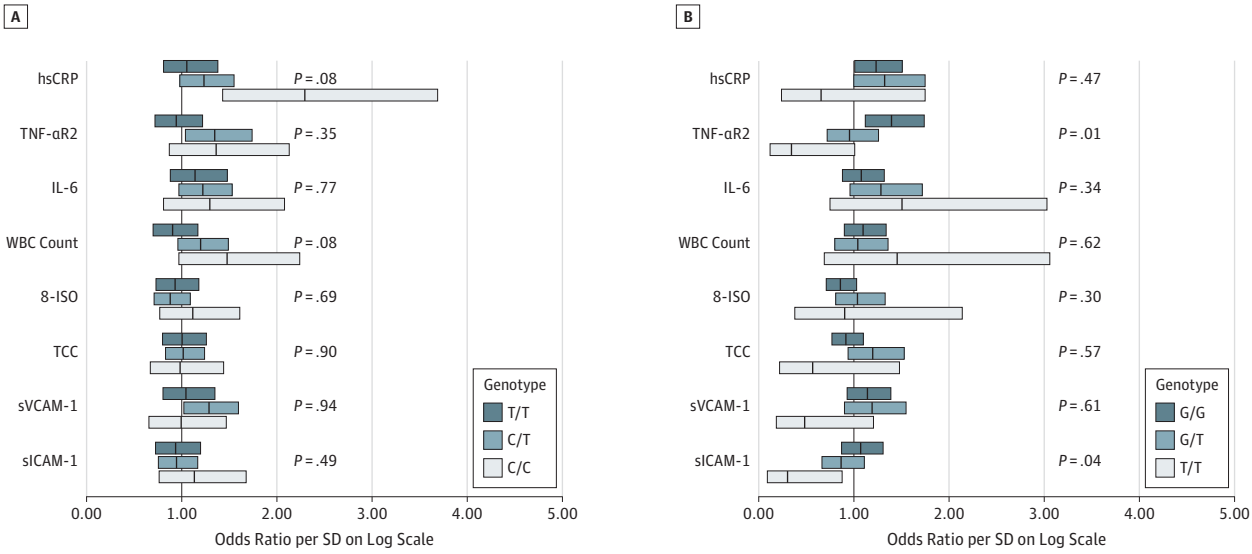
Abbreviations: AMD, age-related macular degeneration; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; 8-ISO, 8-isoprostane; OR, odds ratio; sICAM-1, serum intercellular adhesion molecule–1; sVCAM-1, serum vascular cell adhesion molecule–1; TCC, total carbonyl content; TNF-αR2, tumor necrosis factor–α receptor 2; WBC, white blood cell.

SI conversion factors: To convert hsCRP to nmol/L, multiply by 9.524; and WBC count to $\times 10^9$/L, multiply by 0.001.

[a] Model 1 adjusted for age and sex.

[b] Model 2 adjusted for age, sex, smoking habits, physical activity, statin use, anti-inflammatory medication use, and body mass index.

Figure. Relationship of Markers of Inflammation, Endothelial Dysfunction, and Oxidative Stress to Early AMD Incidence



The graphs show the relationship of markers of inflammation, endothelial dysfunction, and oxidative stress to the 20-year incidence of early age-related macular degeneration in the Beaver Dam Eye Study (1988-1990 to 2008-2010) stratified by complement factor H rs1061170 genotype (A) and age-related maculopathy susceptibility 2 rs10490924 genotype (B). C indicates cytosine; G, guanine; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; 8-ISO, 8-isoprostane; sICAM-1, soluble intercellular adhesion molecule–1; sVCAM-1, soluble vascular cell adhesion molecule–1; T, thymine; TCC, total carbonyl content; TNF-αR2, tumor necrosis factor–α receptor 2; WBC, white blood cell.

and other factors, those with high levels of hsCRP had greater odds of developing early AMD (OR >3 mg/dL vs <1 mg/dL, 1.69; *P* = .04).[45]

The pathogenetic mechanisms underlying the role of hsCRP and other inflammatory biomarkers in the development of AMD are complex and not fully understood.[26] Johnson and colleagues[57] speculated that elevations of hsCRP during acute phase reactions over a lifetime in individuals homozygous for the *CFH* rs1061170 risk alleles resulted in increasing tissue damage to the Bruch membrane and the RPE, further increasing the risk for AMD compared with those homozygous for the wild type of *CFH*. There is emerging evidence that the association of elevated levels of hsCRP with early AMD is not owing to hsCRP directly damaging the RPE and Bruch membrane. Instead, when hsCRP levels increase (eg, during an acute phase reaction), it has been shown that CRP is more likely to bind more strongly to the *CFH* gene site when 1 or 2 risk alleles are present compared with when no risk alleles are present.[26,58,59] The stronger binding of hsCRP is thought to block the regulatory function of *CFH* in deactivating surface-bound C3b, a key factor in the response of the complement immune system to inflammatory stimuli.[58,59] The finding in the BDES of a borderline multiplicative interaction of *CFH* with higher levels of hsCRP for the 20-year cumulative incidence of early AMD when 1 and 2 risk alleles are present is consistent with these observations.

Our study showed that TNF-αR2 was associated with the development of early AMD independent of BMI, smoking sta-

Table 3. Effects of Markers of Inflammation, Endothelial Dysfunction, and Oxidative Stress on the Risk for AMD in Risk-Assessment Models[a]

Marker[b]	Traditional Risk-Factor Model				Traditional Risk Factors + Genetic Factors Model			
	AUC (95% CI)		Change in AUC, %	*P* Value	AUC (95% CI)		Change in AUC, %	*P* Value
	Without Markers in Model	With Markers in Model			Without Markers in Model	With Marker in Model		
hsCRP	0.6714 (0.6212-0.7216)	0.6786 (0.6295-0.7277)	1.07	.42	0.6923 (0.6411-0.7435)	0.6997 (0.6492-0.7502)	1.06	.32
TNF-αR2		0.6715 (0.6217-0.7214)	0.02	.97		0.6926 (0.6417-0.7435)	0.04	.93
IL-6		0.6778 (0.6287-0.7269)	0.95	.47		0.6992 (0.6483-0.7500)	0.98	.38
WBC count		0.6747 (0.6239-0.7255)	0.49	.59		0.6962 (0.6445-0.7479)	0.56	.48
8-ISO		0.6726 (0.6221-0.7232)	0.18	.69		0.6927 (0.6412-0.7443)	0.06	.86
TCC		0.6750 (0.6251-0.7249)	0.54	.67		0.6953 (0.6443-0.7462)	0.42	.65
sVCAM-1		0.6720 (0.6215-0.7226)	0.09	.92		0.6956 (0.6437-0.7474)	0.46	.61
sICAM-1		0.6719 (0.6214-0.7225)	0.08	.82		0.6909 (0.6393-0.7425)	−0.20	.45

Abbreviations: AMD, age-related macular degeneration; AUC, area under the receiver operating characteristic curve; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; 8-ISO, 8-isoprostane; TCC, total carbonyl content; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF-αR2, tumor necrosis factor- α receptor 2; WBC, white blood cell.

SI conversion factors: To convert hsCRP to nmol/L, multiply by 9.524; and WBC count to $\times 10^9$/L, multiply by 0.001.

[a] Traditional risk factors = age, sex, smoking status, physical activity, and body mass index; genetic factors = complement factor H rs1061170 and age-related maculopathy susceptibility 2 rs10490924.

[b] Markers modeled per SD on the logarithmic scale.

tus, and other factors. Their relationship was no longer statistically significant when hsCRP was included in the model. Tumor necrosis factor-α receptor 2 was not previously shown to be related to the prevalence of any AMD or the progression to late AMD.[32,34] Tumor necrosis factor-α is a cytokine involved in cell activation, differentiation, and apoptosis, and it has been shown to be related to AMD in some studies.[60,61] The receptor TNF-αR2 is expressed in the choroid vascular cells, RPE, and Mueller cells in the retina. Its role in the pathogenesis of AMD is poorly understood, as is the reason the relationship was stronger in postmenopausal women than in premenopausal women. The reason for the inverse interaction in the BDES group with *ARMS2* is not understood; it may be a chance finding.

In the BDES, there was no relation of 2 markers of oxidative stress, serum 8-ISO, and TCC to the incidence of AMD. This is consistent with the lack of a protective effect of antioxidant vitamins for the incidence of early AMD in the Age-Related Eye Disease Study 1.[62] The RPE has been shown to be vulnerable to oxidative damage by radical-catalyzed lipid peroxidation.[63-65] The lack of an association may be due to oxidative stress not being related to incident early AMD or that the 2 markers do not reflect oxidative stress occurring at the cellular level at the RPE. The variability of these 2 oxidative stress measures may have affected our ability to find a relationship if it were present. To our knowledge, few other epidemiological studies have examined the relationships of these measures of oxidative stress to AMD. In one, a prospective case-control study involving 77 patients with AMD and 75 control participants, plasma F2 isoprostane was not related to AMD after adjustment for age, sex, and smoking status.[37]

In the BDES, while adjusting for age, sex, smoking status, and other factors, sVCAM-1, but not sICAM-1, was associated with the incidence of early AMD. Both of these cellular adhesion molecules are transmembrane cell surface proteins with immunoglobulin superfamily domains. They regulate inflammation by attracting WBCs and controlling their migration into the blood vessel wall.[66] Increased expression of these molecules in the cellular wall is reflected by increases in soluble forms of these molecules in the plasma. Increases in the number of WBCs have been shown in the choroid of eyes with early and late AMD.[67-69] Complement-mediated activation of choroidal endothelial secretion of sICAM-1 has been hypothesized to play a role in the pathogenesis of AMD.[70] However, few associations were found in the studies that have examined these relationships.[31,32,71]

Our findings suggest that while there are statistically significant, clinically meaningful relationships of the inflammatory markers in persons without AMD, they have limited prognostic value for predicting the incidence of early AMD, independent of age, sex, smoking history, and other traditional risk factors. The increase in area under the receiver operating characteristic curve of 1.02% for inclusion of hsCRP in the risk-prediction model was small and not statistically significant. It compares unfavorably with other potential predictive factors used for other end points (eg, hsCRP and serum high-density lipoprotein cholesterol levels) when added to the Framingham risk score for coronary heart disease in the Atherosclerosis Risk in Communities Study.[72-74]

There were many strengths to our study including the use of standard protocols to measure AMD from fundus photographs during a 20-year period in a representative population-based study. There were also limitations. First, the analyses were performed in a randomized sample of the cohort to minimize bias. It is possible that this sample may not be representative of the cohort. However, randomization appeared to minimize this possibility; there were few differences between those randomized and those not randomized. Additionally, hsCRP and WBC count were measured in the whole cohort at baseline and the findings were similar to those reported in the smaller randomized cohort. Second, selective survival may have obscured relationships if people with high levels of se-

rum 8-ISO or TCC who developed early AMD were more likely to die before being examined than those with low levels of these markers. Those with higher levels of serum 8-ISO were not more likely to die than to be observed with or without early AMD (OR per SD on the logarithmic scale, 0.98; 95% CI, 0.80-1.15; $P = .80$). However, those with higher levels of TCC were more likely to die than to be observed with or without early AMD (OR, 1.22; 95% CI, 1.06-1.38; $P = .02$) after adjusting for age, sex, smoking status, physical activity, BMI, and anti-inflammatory medication use. Third, a single measure of a marker (eg, hsCRP) may not be representative of lifetime exposure. However, Nash and colleagues[75] evaluated the 10-year percentage agreement between groups for levels of IL-6 (50.8%) and hsCRP (53.4%), and their data suggest that the levels of these inflammatory markers track over time and are fairly stable. Data from another study suggest modest variability of inflammatory markers over time, dependent partially on changes in cardiovascular risk factors (eg, obesity, physical activity, and smoking status) as people age.[76] Fourth, the long period between freezing and measurement of samples may have resulted in the greater variability found in serum 8-ISO and TCC levels, reducing our ability to find a relationship. Serum samples were stored at −80°C. These tests were found to be stable with essentially no evidence of auto-oxidation in a pilot study from the Nurses' Health Study.[77] Furthermore, Schwedhelm and colleagues[78] reported long-term storage of blood samples in prospective studies at −80°C to be stable with respect to these markers of oxidative stress.

Conclusions

Inflammatory markers and 1 marker of endothelial dysfunction were modestly associated with the 20-year cumulative incidence of early AMD in the BDES. These data provide further support for the role of inflammation in the pathogenesis of early AMD. It may be 1 of many mechanisms involved in the development of this complex multifactorial disease. It is unknown whether these associations represent a cause and effect relationship or whether other unknown confounders accounted for the findings. Even if inflammatory processes are a cause of early AMD, it is unknown whether interventions that reduce systemic inflammatory processes will reduce the incidence of early AMD.

ARTICLE INFORMATION

Submitted for Publication: June 7, 2013; final revision received September 17, 2013; accepted September 24, 2013.

Published Online: January 30, 2014. doi:10.1001/jamaophthalmol.2013.7671.

Author Affiliations: Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison (Ronald Klein, Myers, Cruickshanks, Danforth, B.E. Klein); Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison (Ronald Klein, Gangnon); Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison (Cruickshanks, Gangnon); Departments of Epidemiology and Biostatistics, and Genetics and Ophthalmology, Case Western Reserve University, Cleveland, Ohio (Sivakumaran, Iyengar); Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio (Sivakumaran); Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis (Tsai).

Author Contributions: Dr R. Klein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Ms Myers and Dr Gangnon conducted and are responsible for the data analysis.
Study concept and design: R. Klein, Cruickshanks.
Acquisition of data: R. Klein, Cruickshanks, Danforth, Sivakumaran, Tsai, B. E. K. Klein.
Analysis and interpretation of data: R. Klein, Myers, Gangnon, Iyengar, B. E. K. Klein.
Drafting of the manuscript: R. Klein.
Critical revision of the manuscript for important intellectual content: Myers, Cruickshanks, Gangnon, Danforth, Sivakumaran, Iyengar, Tsai, B. E. K. Klein.
Statistical analysis: Myers, Gangnon, Iyengar.
Obtained funding: R. Klein, Cruickshanks, Iyengar, B. E. K. Klein.
Administrative, technical, and material support: Danforth.
Study supervision: R. Klein.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported by National Institutes of Health grant EY06594 (Drs B. E. K. Klein and R. Klein), National Institute on Aging grant AG11099 (Dr Cruickshanks), and National Institute of Diabetes and Digestive and Kidney Diseases grant DK073217, National Institutes of Health, as well as, in part, by Research to Prevent Blindness, New York, New York. The National Eye Institute and National Institute of Diabetes and Digestive and Kidney Diseases provided funding for this study including collection and analyses of data; Research to Prevent Blindness provided additional support for data analyses.

Role of the Sponsor: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclaimer: The content of this study is solely the responsibility of the authors and does not necessarily reflect the official views of the National Eye Institute or the National Institutes of Health.

Correction: This article was corrected online February 4, 2014, for an incorrect *P* value in the Results section.

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