Association of Polymorphisms in the *CRP* Gene With Circulating C-Reactive Protein Levels and Cardiovascular Events

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Context C-reactive protein (CRP) is an inflammation protein that may play a role in the pathogenesis of cardiovascular disease (CVD).

Objective To assess whether polymorphisms in the *CRP* gene are associated with plasma CRP, carotid intima-media thickness (CIMT), and CVD events.

Design, Setting, and Participants In the prospective, population-based Cardiovascular Health Study, 4 tag single-nucleotide polymorphisms (SNPs) (1919A/T, 2667G/C, 3872G/A, 5237A/G) were genotyped in 3941 white (European American) participants and 5 tag SNPs (addition of 790A/T) were genotyped in 700 black (African American) participants, aged 65 years or older, all of whom were without myocardial infarction (MI) or stroke before study entry. Median follow-up was 13 years (1989-2003).

Main Outcome Measures Baseline CIMT; occurrence of MI, stroke, and CVD mortality during follow-up.

Results In white participants, 461 incident MIs, 491 incident strokes, and 490 CVD-related deaths occurred; in black participants, 67 incident MIs, 78 incident strokes, and 75 CVD-related deaths occurred. The 1919T and 790T alleles were associated with higher CRP levels in white and black participants, respectively. The 3872A allele was associated with lower CRP levels in both populations, and the 2667C allele was associated with lower CRP levels in white participants only. There was no association between CIMT and any *CRP* gene polymorphism in either population. In white participants, the 1919T allele was associated with increased risk of stroke for TT vs AA (hazard ratio [HR], 1.40; 95% confidence interval [CI], 1.06-1.87) and for CVD mortality (HR, 1.40; 95% CI, 1.10-1.90). In black participants, homozygosity for the 790T allele was associated with a 4-fold increased risk of MI compared with homozygosity for the 790A allele (95% CI, 1.58-10.53). The minor alleles of the 2 SNPs associated with lower plasma CRP concentration in white participants (2667C and 3872A) were associated with decreased risk of CVD mortality.

Conclusions Genetic variation in the *CRP* gene is associated with plasma CRP levels and CVD risk in older adults.

JAMA. 2006;296:2703-2711 www.jama.com

C-REACTIVE PROTEIN (CRP), AN acute phase reactant, plays an important role in acute and chronic inflammation. Inflammation contributes to all phases of atherosclerosis, from fatty streak initiation to cardiovascular disease (CVD) events.[1] Plasma CRP levels measured at a single point predict future incident CVD events, such as myocardial infarction (MI) and stroke,[2-7] leading to interest in the use of CRP as a biomarker of CVD risk.[8] Although some data from in vitro observations and experimental models support a role of CRP in atherogenesis,[9] the direct involvement of CRP remains in question.

Plasma CRP levels are under genetic influence.[10,11] In healthy, young white

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(European American) and black (African American) adults, *CRP* gene haplotypes have been shown to be strongly associated with plasma concentration.[12-14] This led us to hypothesize that CRP is causally involved in atherosclerosis, and that alleles associated with higher CRP would confer an increased risk of CVD. Like the variant in *PCSK9* (proprotein convertase subtilisin/kexin type 9), which was associated not only with lifelong lowering of low-density lipoprotein cholesterol but also with a reduced risk of CVD, *CRP* gene variants that affect CRP concentration levels may reflect lifetime exposure to CRP more accurately than CRP serum concentrations measured at a single point in time.[15]

METHODS

Cardiovascular Health Study

The Cardiovascular Health Study (CHS) design, recruitment, and data collection have been previously described.[16] There were 5888 men and women (≥65 years) recruited from 4 US field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. Race/ethnicity was defined by self-report. The original cohort (n=5201) was recruited from 1989 to 1990. A second cohort (n=687) of black participants was recruited from 1992 to 1993. All participants were followed up through 2003, unless lost to follow-up, dropped out of study, or had an event before that time. Only individuals who reported race as white or black were included in our study; we excluded participants with prior MI or stroke (n=765), or for whom DNA was not collected or who did not consent to the use of their DNA (n=482). All procedures were conducted under institutionally approved protocols for use of human subjects, and all participants provided written informed consent.

The CHS baseline evaluation included demographic, lifestyle, and medical histories, physical examination, and fasting blood collection.[16,17] Common carotid artery wall intima-media thickness (CIMT) was determined at the baseline examination by high-resolution B-mode ultrasonography.[18] The mean maximal intima-media thickness of the common or internal carotid wall was calculated from 4 scans on each carotid artery (right and left side) for each participant. C-reactive protein was measured on stored EDTA plasma from the baseline examination by using a high-sensitivity enzyme-linked immunosorbent assay (coefficient of variation, 6.2%).[19] Plasma CRP was also measured 3 years after the baseline examination on a subset of 3256 white participants by immunonephelometry (coefficient of variation, 3.0%; BN-II instrument, Dade-Behring, Deerfield, Ill). Both the enzyme-linked immunosorbent assay and nephelometric assays are standardized according to the World Health Organization reference plasma and yield highly correlated values.

Definition of Clinical CVD Events

Details of event ascertainment in CHS have been previously published.[20] Participants, family members, or other previously identified informants reported new cardiovascular events during semiannual contacts. Medical records were reviewed, and events were adjudicated by a physician review panel. Criteria for MI included history of chest pain, cardiac enzyme levels, and characteristic changes on serial electrocardiograms. Criteria for stroke included onset of symptoms, duration of deficits, and findings on computed tomography or magnetic resonance imaging. Adjudicated events occurring through June 30, 2003, were available. Participants were followed up for up to 14 years (median: 13 years for white cohort and 10 years for black cohort).

Primary clinical end points for this analysis were incident non-procedure–related fatal or nonfatal MI, stroke, and CVD mortality, defined as fatal event in which death was adjudicated due to atherosclerotic coronary heart disease or cerebrovascular disease, including definite fatal MI, definite fatal stroke, and definite or probable fatal coronary heart disease.[20]

CRP Polymorphism Genotyping

Based on linkage disequilibrium and haplotype patterns across the *CRP* gene locus,[12] we selected tag single-nucleotide polymorphisms (SNPs) for genotyping. The SNP numbering is relative to GenBank accession AF449713. Four tag SNPs (1919 [rs1417938], located in intron 1; 2667 [rs1800947], a synonymous SNP located in exon 2; and 3872 [rs1205] and 5237 [rs2808630], both located in the 3′ flanking region) were typed in both the white and black CHS samples. A fifth tag SNP, 790 (rs3093058), located in the 5′ promoter region is prevalent only among black participants, and therefore was genotyped only in the black CHS samples. Tag SNPs were genotyped using TaqMan Assays By Design (Applied Biosystems, Foster City, Calif) under standard conditions. Probe and primer sequences are available from the authors by request.

Statistical Analysis

Each of the *CRP* polymorphisms was assessed to determine if the observed genotype frequencies were consistent with expected Hardy-Weinberg proportions by using Pearson χ^2 tests. Marker-marker linkage disequilibrium was assessed using Lewontin's D′ statistic[21] and the squared correlation statistic Δ^2.[22] We considered statistical significance at the α=.05 level, before and after adjustment for multiple tests.

Genotype Association. All analyses were stratified by ethnicity to minimize potential confounding due to population stratification. Non-normal distributions were log-transformed as necessary. To assess SNP associations with plasma CRP levels and CIMT, we used analysis of covariance models (SAS version 8.0; SAS Institute, Cary, NC). Covariate adjustment was made for age, sex, clinic site, body mass index (calculated as weight in kilo-

grams divided by height in meters squared), smoking status, triglycerides, and clinical or subclinical (for CRP only) disease at baseline.[23] Genotypes were tested for general association (no mode-of-inheritance assumption) using a 2-*df* F test. Covariate-adjusted mean values (least square means) were calculated from the regression coefficients.

We performed Cox proportional hazards regression models to test for association between SNPs and risk of incident MI, stroke, or CVD mortality. Two *df* likelihood ratio tests were conducted to test for a general association between genotype and time-to-CVD event. Cox proportional hazards regression models were performed with 3 levels of covariate adjustment: (1) adjustment for baseline age, sex, clinic site, body mass index, systolic blood pressure, diabetes mellitus, hypertension, and smoking status; (2) level 1 plus additional adjustment for baseline CRP concentration; and (3) level 2 plus additional adjustment for 3-year CRP concentration. To control for population stratification in black participants, we also adjusted black regression models for a variable that reflects the estimated proportion of African ancestry for each individual.[24]

Haplotype Association. Associations between haplotypes and plasma CRP and CIMT were assessed using an expectation-maximization–derived score test,[25] implemented in HAPLO.STAT (http://www.mayo.edu/statgen). For time-to-event data, we applied a method that formulates the effects of haplotypes and environmental variables on the time-to-disease occurrence in a Cox proportional hazards regression model by using an expectation-maximization–based algorithm to maximize the likelihood for the relative risks and other parameters.[26] Both methods provide a global test of haplotype association and tests of association for individual haplotypes. An additive genetic mechanism was assumed. Only haplotypes with estimated frequencies of more than 5% were included. HAPLO.STAT performs individual haplotype tests that compare each haplotype to all other haplotypes pooled together, whereas the Cox proportional hazards regression model approach compares each haplotype to the most common haplotype.

Table 1. Participant Characteristics at Baseline by Ethnicity*

Characteristic	White Participants (n = 3941)	Black Participants (n = 700)
Age, mean (SD), y	72.6 (5.5)	72.7 (5.5)
Women	2346 (59.3)	448 (64.0)
BMI, mean (SD)	26.3 (4.5)	28.6 (5.5)
Diabetes mellitus†	516 (13.1)	157 (22.6)
Current smoker‡	431 (10.9)	114 (16.4)
Hypertension§	2150 (54.7)	517 (73.9)
Blood pressure, mean (SD), mm Hg		
Systolic	135.4 (21.3)	142.6 (22.5)
Diastolic	70.0 (11.4)	76.4 (11.3)
Cholesterol, mean (SD), mg/dL		
Total	212.3 (38.9)	209.5 (37.9)
HDL	54.6 (15.8)	58.3 (15.4)
LDL	130.1 (35.6)	128.6 (34.9)
CRP, median (IQR), mg/L‖	2.30 (1.19-4.09)	5.89 (1.59-6.61)
Carotid intima-media thickness, mean (SD), mm	0.99 (0.20)	1.11 (0.22)
Incident CVD events		
Myocardial infarction	559 (14.2)	95 (13.6)
Stroke	562 (14.3)	92 (13.1)
CVD mortality	586 (14.9)	95 (13.6)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.
SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259.
*Data are presented as number (percentage) unless otherwise indicated.
†Classified according to the American Diabetes Association guidelines.
‡Smoking behavior was ascertained by self-report. Current smokers smoked cigarettes during the 30 days preceding examination and had smoked more than 100 cigarettes or had 5 pack-years of cigarette smoking during their lifetime.
§Defined as seated systolic blood pressure of at least 140 mm Hg, diastolic pressure of at least 90 mm Hg, or self-reported high blood pressure and use of antihypertensive medication.
‖Median (IQR) are presented for plasma CRP concentration because of skewed distribution.

RESULTS

Sample descriptions, including clinical end points, are shown in TABLE 1. Black participants had a higher prevalence of diabetes mellitus, current smoking, and higher median baseline plasma CRP compared with white participants.

The observed genotype distributions for the 5 *CRP* tag SNPs (TABLE 2) were consistent with Hardy-Weinberg equilibrium. There was little evidence of historical recombination between SNPs (estimated D′ range, 0.98-1.00 in white participants and 0.85-1.00 in black participants). Because the SNPs were selected to maximize linkage disequilibrium coverage across the *CRP* gene, there was only a modest degree of correlation in genotype data between SNPs (estimated Δ^2 range, 0.03-0.22 in white participants and 0.00-0.06 in black participants).

CRP Genotype, Plasma CRP Level, and CIMT

Three of 4 SNPs in the white sample and 2 of 5 SNPs in the black sample were strongly associated with baseline CRP concentration (Table 2). The estimated proportion of variation in plasma CRP level explained by significant single SNP genotypes ranged from 0.7% to 1.8% in white participants and 1.2% to 4.4% in black participants. The estimated proportion of variation explained when including all *CRP* SNPs was 2.6% in white participants and 6.4% in black participants. Similar results were obtained using plasma CRP measurements obtained after 3 years of fol-

low-up (data available from authors). Haplotype-based analyses of plasma CRP levels yielded results that were consistent with single SNP results (TABLE 3). There was no association between CIMT and any *CRP* SNP genotype or haplotype in either the white or black samples.

CRP Genotype and Risk of Clinical CVD Events

Among white participants, SNPs 1919, 2667, and 3872 were associated with CVD mortality, and SNP 1919 was associated with increased risk for stroke (TABLE 4). In black participants, SNPs 790 and 1919 were associated with incident MI and SNP 790 was associated with incident stroke (TABLE 5). To assess the overall significance of the association between *CRP* SNPs and the risk of clinical CVD events, we performed joint association analyses that included all SNPs simultaneously. Specifically, we conducted likelihood-ratio tests for Cox proportional hazards regression models, in each case comparing the model with all genotyped SNPs to the null hypothesis model that contained no SNPs. The association between all SNPs and CVD mortality in white participants ($P=.004$) and MI in black participants ($P=.005$) were statistically significant at an overall $\alpha=.05$ level, even after accounting for the 3 events (Bonferroni significance threshold $P=.05$ divided by 3: $P=.02$). The association between all SNPs and stroke in black participants ($P=.04$) was statistically significant before, but not after, accounting for 3 events.

Adjusting for Plasma CRP Level

In this study, CRP level predicted CVD risk.[7] Because *CRP* genotype likely influences risk through CRP synthesis, we additionally adjusted for plasma CRP level, which resulted in only a modest attenuation of hazard ratios (Table 4 and Table 5). The CVD risk estimates remained essentially unchanged when we limited analyses to the 3256 white participants with 2 plasma CRP measurements, and incorporated both measurements into the regression models (data available from authors).

Table 2. Association Between *CRP* Tag SNP Genotype and Plasma CRP Concentration and CIMT by Ethnicity*

	White Participants			Black Participants		
		Least Square Means (95% CI)			Least Square Means (95% CI)	
CRP Polymorphism	No.	Log (CRP+1)	Log CIMT	No.	Log (CRP+1)	Log CIMT
790 A/T†						
AA				483	0.173 (0.082 to 0.265)	0.730 (0.714 to 0.747)
AT				185	0.434 (0.324 to 0.543)	0.740 (0.719 to 0.760)
TT				19	0.402 (0.151 to 0.653)	0.774 (0.727 to 0.821)
P value‡					<.001§	.12
1919 A/T						
AA	1874	0.077 (0.052 to 0.101)	0.688 (0.684 to 0.692)	508	0.248 (0.155 to 0.341)	0.734 (0.717 to 0.751)
AT	1673	0.134 (0.109 to 0.160)	0.689 (0.685 to 0.693)	162	0.207 (0.090 to 0.324)	0.734 (0.713 to 0.756)
TT	357	0.222 (0.167 to 0.277)	0.683 (0.674 to 0.692)	16	0.473 (0.195 to 0.751)	0.722 (0.672 to 0.772)
P value‡		<.001§	.48		.16	.88
2667 G/C						
GG	3390	0.136 (0.118 to 0.155)	0.687 (0.684 to 0.690)	664	0.254 (0.163 to 0.344)	0.733 (0.717 to 0.749)
CG	499	–0.030 (–0.077 to 0.017)	0.693 (0.685 to 0.700)	23	0.121 (–0.121 to 0.363)	0.756 (0.712 to 0.799)
CC	17	0.050 (–0.203 to 0.304)	0.673 (0.632 to 0.714)	0		
P value‡		<.001§	.33		.27	.29
3872 G/A						
GG	1693	0.192 (0.167 to 0.218)	0.690 (0.686 to 0.694)	443	0.299 (0.204 to 0.394)	0.734 (0.717 to 0.751)
AG	1752	0.083 (0.058 to 0.108)	0.686 (0.682 to 0.690)	215	0.195 (0.087 to 0.303)	0.739 (0.720 to 0.759)
AA	460	–0.052 (–0.101 to 0.004)	0.687 (0.678 to 0.695)	29	0.038 (–0.165 to 0.241)	0.711 (0.674 to 0.749)
P value‡		<.001§	.46		.005‖	.33
5237 A/G						
AA	2059	0.112 (0.088 to 0.135)	0.685 (0.682 to 0.689)	459	0.258 (0.165 to 0.351)	0.736 (0.719 to 0.753)
AG	1568	0.114 (0.088 to 0.141)	0.691 (0.686 to 0.695)	206	0.244 (0.134 to 0.354)	0.730 (0.710 to 0.750)
GG	280	0.142 (0.079 to 0.205)	0.693 (0.683 to 0.703)	22	–0.042 (–0.282 to 0.198)	0.721 (0.677 to 0.764)
P value‡		.33	.13		.04	.65

Abbreviations: CI, confidence interval; CIMT, carotid intima-media thickness; CRP, C-reactive protein; SNP, single-nucleotide polymorphism.
*Baseline CRP level analyses were adjusted for age, sex, clinic site, body mass index, current smoking status, triglycerides, and clinical or subclinical cardiovascular disease (CVD) at baseline. The CIMT analyses were adjusted for age, sex, clinic site, body mass index, current smoking status, triglycerides, and clinical CVD at baseline. Analyses for black participants are additionally adjusted for estimated genetic ancestry to control for population stratification.[24]
†The 790 SNP was not genotyped in white participants.
‡Unadjusted for multiple tests.
§Statistically significant at an overall $\alpha = .0001$ level after applying a Bonferroni correction to account for results from all analyzed SNPs.
‖Statistically significant at an overall $\alpha = .05$ level after applying a Bonferroni correction to account for results from all analyzed SNPs.

Table 3. Association Between CRP Haplotypes and Plasma CRP Concentration and CIMT, Stratified by Ethnicity*

	Allele						Log CRP		Log CIMT	
Haplotype	790 A/T SNP	1919 A/T SNP	2667 G/C SNP	3872 G/A SNP	5237 A/G SNP	Estimated Frequency, %	Score†	*P* Value‡	Score†	*P* Value‡
White participants										
E1		A	C	A	A	6.7	−7.03	<.001	0.22	.84
E2		A	G	A	A	27.2	−6.71	<.001	−1.56	.12
E3		A	G	G	G	27.6	1.38	.17	2.11	.03
E4		T	G	G	A	30.2	5.09	<.001	−0.17	.86
E5		A	G	G	A	8.1	6.63	<.001	−0.81	.41
Global								<.001		.33
Black participants										
A2	A	A	G	A	A	18.0	−3.73	<.001	0.87	.38
A3	A	A	G	G	G	18.8	−1.66	.10	0.75	.46
A4	A	T	G	G	A	13.9	−0.58	.56	−1.09	.28
A5	A	A	G	G	A	31.3	0.47	.64	0.96	.34
A6	T	A	G	G	A	16.6	5.94	<.001	1.26	.21
Global								<.001		.40

Abbreviations: CIMT, carotid intima-media thickness; CRP, C-reactive protein; SNP, single-nucleotide polymorphism.
*White participants comprised European Americans (E1 thru E5) and black participants comprised African Americans (A2 thru A6).
†HAPLO.STAT (http://www.mayo.edu/statgen) program score statistic.
‡Permutation-based score test results. Unadjusted *P* values for individual haplotypes represent the statistical significance of testing whether the mean CRP levels are the same for the particular haplotype compared with the CRP levels over all other haplotypes combined. The global *P* value corresponds to testing the null hypothesis that CRP levels are the same for all haplotypes. C-reactive protein levels and CIMT are adjusted for age, sex, clinic site, body mass index, triglycerides, current smoking status, and clinical cardiovascular disease (CVD) at baseline. C-reactive protein levels are additionally adjusted for subclinical CVD at baseline. Analyses for black participants are additionally adjusted for estimated genetic ancestry to control for population stratification.[24] Haplotypes with estimated frequency of less than 5% were excluded from analysis.

Table 4. Association Between *CRP* Tag SNP Genotype and Risk of CVD Events in White Participants Before and After Adjustment for Plasma CRP Concentration*

	Hazard Ratio (95% CI)					
	Myocardial Infarction (Events = 461)		Stroke (Events = 491)		CVD Mortality (Events = 490)	
CRP Polymorphism	Before Plasma CRP Adjustment	After Plasma CRP Adjustment	Before Plasma CRP Adjustment	After Plasma CRP Adjustment	Before Plasma CRP Adjustment	After Plasma CRP Adjustment
1919 A/T						
AA (n = 1874)	1.00	1.00	1.00	1.00	1.00	1.00
AT (n = 1673)	1.04 (0.88-1.24)	1.04 (0.87-1.24)	1.28 (1.07-1.52)	1.27 (1.06-1.51)	1.23 (1.04-1.46)	1.23 (1.04-1.46)
TT (n = 357)	1.10 (0.82-1.47)	1.05 (0.78-1.42)	1.40 (1.06-1.87)	1.38 (1.03-1.84)	1.40 (1.10-1.90)	1.36 (1.03-1.79)
P value†	.79	.90	.008‡	.01‡	.009‡	.02
2667 G/C						
GG (n = 3390)	1.00	1.00	1.00	1.00	1.00	1.00
CG (n = 499)	0.97 (0.75-1.26)	1.00 (0.77-1.29)	0.97 (0.75-1.25)	0.98 (0.76-1.27)	0.65 (0.48-0.88)	0.67 (0.50-0.90)
CC (n = 17)	0.86 (0.21-3.44)	0.85 (0.21-3.40)	0.74 (0.18-2.97)	0.71 (0.18-2.85)	1.69 (0.63-4.54)	1.50 (0.56-4.04)
P value†	.96	.97	.98	.88	.01‡	.02
3872 G/A						
GG (n = 1693)	1.00	1.00	1.00	1.00	1.00	1.00
AG (n = 1752)	0.86 (0.72-1.02)	0.88 (0.74-1.05)	0.94 (0.79-1.12)	0.95 (0.80-1.13)	0.93 (0.78-1.10)	0.94 (0.80-1.12)
AA (n = 460)	0.84 (0.64-1.11)	0.87 (0.66-1.15)	0.70 (0.52-0.95)	0.71 (0.53-0.97)	0.65 (0.48-0.88)	0.67 (0.49-0.91)
P value†	.17	.30	.07	.09	.02	.04
5237 A/G						
AA (n = 2059)	1.00	1.00	1.00	1.00	1.00	1.00
AG (n = 1568)	1.19 (1.00-1.42)	1.19 (1.00-1.41)	1.04 (0.87-1.23)	1.04 (0.88-1.24)	1.08 (0.91-1.27)	1.08 (0.92-1.29)
GG (n = 280)	0.94 (0.67-1.33)	0.93 (0.66-1.31)	0.92 (0.59-1.27)	0.92 (0.66-1.28)	0.74 (0.53-1.05)	0.76 (0.53-1.07)
P value†	.10	.10	.75	.74	.11	.12

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; SNP, single-nucleotide polymorphism.
*Hazard ratios are adjusted for sex, clinic site, and baseline measures of age, body mass index, systolic blood pressure, diabetes mellitus, hypertension, and current smoking status. *P* values are for general test of association (no mode of inheritance). Participants with a myocardial infarction or stroke before baseline were excluded from analyses.
†Unadjusted for multiple tests.
‡Statistically significant at an overall $\alpha = .05$ level after applying a Bonferroni correction to account for results from the 4 analyzed SNPs.

Haplotype-Based Analyses of Clinical CVD Events

Haplotype-based analyses gave results similar to the single SNP analyses in both white and black participants (TABLE 6). Statistically significant (event-specific) haplotype-based associations were observed for 3 (MI risk in black participants; stroke risk and CVD mortality in white participants) of the 6 ethnicity-event combinations and a trend was observed for a fourth event (stroke risk in black participants). The haplotype-based association with CVD mortality in white participants remained statistically significant ($P<.05$) after applying a Bonferroni correction to account for multiple testing with respect to the 3 events (white participants).

COMMENT

Our results suggest that a genetic basis may underlie, in part, the relationship between CRP concentration and CVD risk in older adults. The association we observed between *CRP* tag SNP genotype and plasma CRP concentration confirms previous findings in younger adults.[12-14,27] Together these results provide strong evidence that genetic variation within the *CRP* gene influences plasma CRP levels over the adult lifespan.

We additionally observed associations between *CRP* SNP genotypes and risk of future CVD events. Specifically, the 790 T allele was associated with increased risk of MI in black participants, the 1919 T allele was associated with increased risk of stroke and CVD mortality in white participants, and the minor alleles of 2667 and 3872 SNPs were associated with decreased risk of CVD mortality in white participants. The direction of the CVD risk estimates tended to be consistent with associations with plasma CRP concentration measured late in life. The specific CVD outcomes associated with *CRP* genotype differed by ethnicity. Possible explanations include differences in allele frequencies between white and black participants, reduced statistical power in the smaller black cohort, or chance association. In contrast with the observed association with clinical CVD, there was no evidence of association between the same *CRP* gene variants and

Table 5. Association Between *CRP* Tag SNP Genotype and Risk of CVD Events in Black Participants Before and After Adjustment for Plasma CRP Concentration*

	Hazard Ratios (95% CI)					
	Myocardial Infarction (Events = 67)		Stroke (Events = 78)		CVD Mortality (Events = 75)	
CRP Polymorphism	Before Plasma CRP Adjustment	After Plasma CRP Adjustment	Before Plasma CRP adjustment	After Plasma CRP Adjustment	Before Plasma CRP Adjustment	After Plasma CRP Adjustment
790 A/T						
AA (n = 483)	1.00	1.00	1.00	1.00	1.00	1.00
AT (n = 185)	1.31 (0.78-2.22)	1.23 (0.72-2.08)	1.45 (0.92-2.28)	1.45 (0.91-2.31)	0.85 (0.53-1.38)	0.83 (0.51-1.34)
TT (n = 19)	4.08 (1.58-10.53)	4.06 (1.57-10.47)	3.64 (1.41-9.39)	3.66 (1.40-9.55)	1.79 (0.64-5.03)	1.73 (0.61-4.86)
P value†	.01	.02	.02	.02	.40	.39
1919 A/T						
AA (n = 508)	1.00	1.00	1.00	1.00	1.00	1.00
AT (n = 162)	0.43 (0.20-0.91)	0.44 (0.21-0.92)	0.65 (0.38-1.12)	0.65 (0.38-1.11)	1.01 (0.62-1.65)	1.01 (0.62-1.65)
TT (n = 16)	2.52 (0.84-7.62)	2.44 (0.81-7.38)	1.41 (0.53-3.78)	1.41 (0.53-3.79)	1.85 (0.62-5.53)	1.79 (0.60-5.40)
P value†	.01	.02	.20	.19	.54	.58
2667 G/C						
GG (n = 664)	1.00	1.00	1.00	1.00	1.00	1.00
CG (n = 23)	1.05 (0.31-5.48)	1.10 (0.26-4.56)	0.31 (0.04-2.25)	0.31 (0.04-2.26)	1.04 (0.25-4.27)	1.06 (0.26-4.38)
CC (n = 0)						
P value†	.94	.90	.25	.25	.96	.94
3872 G/A						
GG (n = 443)	1.00	1.00	1.00	1.00	1.00	1.00
AG (n = 215)	0.54 (0.30-0.98)	0.55 (0.31-1.01)	1.33 (0.86-2.05)	1.35 (0.87-2.09)	1.20 (0.77-1.87)	1.21 (0.77-1.89)
AA (n = 29)	0.37 (0.05-2.70)	0.40 (0.05-2.94)	. . . ‡	. . . ‡	1.72 (0.61-4.86)	1.78 (0.63-5.04)
P value†	.09	.11	.20	.19	.48	.44
5237 A/G						
AA (n = 459)	1.00	1.00	1.00	1.00	1.00	1.00
AG (n = 206)	0.66 (0.38-1.15)	0.66 (0.38-1.15)	0.91 (0.57-1.45)	0.91 (0.57-1.46)	0.68 (0.42-1.10)	0.68 (0.42-1.11)
GG (n = 22)	1.44 (0.51-4.05)	1.63 (0.58-4.63)	1.88 (0.74-4.77)	1.90 (0.74-4.84)	1.50 (0.63-3.58)	1.61 (0.67-3.90)
P value†	.23	.19	.35	.34	.16	.14

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; SNP, single-nucleotide polymorphism.
*Hazard ratios are adjusted for sex, clinic site, and baseline measures of age, body mass index, systolic blood pressure, diabetes mellitus, hypertension, current smoking status, and estimated genetic ancestry to control for population stratification. Participants with a myocardial infarction or stroke before baseline were excluded from analyses. *P* values are for a general test of association (no mode of inheritance).
†Unadjusted for multiple tests.
‡Ellipses indicate no stroke events occurred in the AA genotype for this SNP during the follow-up period.

CIMT, a measure of extent of subclinical atherosclerotic disease, consistent with lack of association of plasma CRP levels with this same measurement.[28,29]

The direction of the observed CRP genotype/haplotype–phenotype associations are also supported by previous functional data on polymorphisms within the *CRP* promoter region.[12,13] The common *CRP* promoter haplotype in black participants tagged by the 790 T allele was associated with increased basal CRP transcriptional activity in human hepatocytes in vitro.[12] Moreover, the 790 T allele and the 1919 T allele (located within the first intron) are both closely linked with high-activity *CRP* haplotype groups that are defined by another common promoter polymorphism at site 1440.[12] In contrast, the minor alleles of the 2667 and 3872 polymorphisms are linked with *CRP* haplotypes associated with decreased promoter activity.

C-reactive protein may be both a marker of CVD and an active participant in the disease process.[30] It is plausible that *CRP* genotype influences CRP synthesis, which in turn could mediate the onset of clinical CVD events. The association between *CRP* genotype and clinical CVD, together with the absence of association between *CRP* genotype and CIMT, suggests a greater involvement of CRP in the transition from subclinical to clinical disease than in atherosclerosis progression. This hypothesis is also consistent with the observation that CRP levels predict risk of stroke in CHS independently of CIMT.[5] C-reactive protein is present in atherosclerotic plaques[31] and has been associated both experimentally and clinically with plaque instability and thrombosis,[32-34] which might increase the propensity toward acute ischemic events, such as MI or stroke. C-reactive protein may be capable of maintaining a procoagulant phenotype through induction of tissue factor and plasminogen activator inhibitor 1 expression in vascular cells or blood monocytes.[35] The complement-dependent ability of CRP to enhance ischemia-induced myocardial tissue damage[36] and cerebral infarct size[37] is another potential mechanism linking CRP and CVD events.

We found that the hazard ratios of CVD for *CRP* genotypes were only slightly attenuated on adjustment for plasma CRP concentration. If the mechanism of the association between CVD events and *CRP* genotype was solely through the gene's influence on CRP concentration, we might expect the attenuation to be more pronounced. However, within our study, individual *CRP* SNP genotypes account for less than 2% of the inter-individual variation in plasma CRP concentration. It may not be that surprising that both CRP concentration and *CRP* genotype are independently associated with CVD events. Moreover, if chronic inflammation plays a causal role in the development of CVD, plasma CRP measured at a single point in time may not adequately reflect an individual's cumulative inflammatory burden. It is known that there is significant intraindividual and analytical variability in plasma CRP levels.[19,38]

It also remains remotely possible that the genetic variant responsible for the *CRP* genotype–CVD event association lies outside of the *CRP* gene locus and that the observed association is not mediated by plasma CRP levels. The low level of linkage disequilibrium between the structural *CRP* gene tran-

Table 6. Association Between CRP Haplotypes and Cardiovascular Events, Stratified by Ethnicity*

	Allele						Myocardial Infarction		Stroke		CVD Mortality	
	790 A/T SNP	1919 A/T SNP	2667 G/C SNP	3872 G/A SNP	5237 A/G SNP	Estimated Frequency, %	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value	RR (95% CI)	*P* Value
White participants												
E1		A	C	A	A	6.8	0.93 (0.72-1.21)	.58	0.82 (0.63-1.06)	.13	0.65 (0.50-0.85)	.002
E2		A	G	A	A	26.9	0.89 (0.76-1.04)	.15	0.80 (0.68-0.94)	.007	0.81 (0.70-0.95)	.009
E3		A	G	G	G	27.7	1.01 (0.86-1.19)	.87	0.87 (0.75-1.02)	.08	0.85 (0.73-0.99)	.03
E4		T	G	G	A	30.4	1.00		1.00		1.00	
E5		A	G	G	A	8.2	0.97 (0.78-1.22)	.83	0.74 (0.58-0.96)	.02	0.91 (0.72-1.14)	.40
Global								.62		.03		.009
Black participants												
A1	A	A	C	A	A	1.6	0.82 (0.17-4.05)	.81	0.36 (0.05-2.82)	.33	1.16 (0.25-5.35)	.85
A2	A	A	G	A	A	19.0	0.44 (0.23-0.85)	.02	1.20 (0.73-1.99)	.47	1.35 (0.85-2.15)	.21
A3	A	A	G	G	G	18.3	0.69 (0.43-1.13)	.14	1.27 (0.80-2.02)	.31	1.03 (0.66-1.61)	.90
A4	A	T	G	G	A	14.8	0.65 (0.37-1.13)	.13	1.03 (0.64-1.67)	.89	1.25 (0.77-2.04)	.37
A5	A	A	G	G	A	30.8	1.00		1.00		1.00	
A6	T	A	G	G	A	15.6	1.25 (0.81-1.95)	.32	1.77 (1.13-2.78)	.01	1.11 (0.71-1.76)	.64
Global								.03		.10		.80

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; RR, relative risk; SNP, single-nucleotide polymorphism.
*White participants comprised European Americans (E1 thru E5) and black participants comprised African Americans (A1 thru A6). Haplotype-specific unadjusted *P* values are for each haplotype compared with the most frequent haplotype as baseline. The global test is the 4 (for white participants) or 5 (for black participants) *df* likelihood ratio test for any haplotype effect. All tests were adjusted for sex, clinic site, and baseline measures of age, body mass index, systolic blood pressure, diabetes mellitus, hypertension, and current smoking status. Analyses for black participants were additionally adjusted for estimated genetic ancestry to control for population stratification.[24]

script and either flanking gene (serum amyloid P gene or the dual-specificity kinase 23 locus) observed in the HapMap data makes it unlikely that the genetic variant responsible for the *CRP* genotype–CVD event association lies outside the *CRP* gene locus. Thus, we consider it substantially more likely that the observed association is mediated directly through the *CRP* gene than through a linked secondary locus. Finally, the current findings imply that the interpretation of data from *CRP* genotype–CVD association studies with respect to mendelian randomization and causality of CRP levels may prove to be more complex than previously appreciated.[39-41]

Although *CRP* genotype was strongly associated with plasma CRP concentration, there was little association between *CRP* genotype and risk of MI or stroke in the Physician's Heath Study, the Framingham Heart Study, or the Rotterdam Study.[14,27,42,43] There are a number of possible explanations for the differences in *CRP* genotype–clinical CVD association between CHS and these other studies. Our analysis included a larger sample size and greater numbers of clinical events; therefore, we had greater power to detect association. In CHS, the association between *CRP* genotype and CVD risk appeared to be strongest for fatal events. In the Physician's Health Study and the Rotterdam Study, the results were not examined according to fatal vs nonfatal outcomes. It is possible that through an effect on acute phase response *CRP* genotype is more strongly associated with more severe events. This concept is supported by a recent study performed on 1827 white patients with acute coronary syndrome, in which *CRP* genotype was observed to be strongly associated with CRP levels measured within 10 days of an event.[44] In addition, the participants in the Physician's Health Study were younger and 100% were men. Although it is generally expected that the influence of genetic factors is more important in younger individuals, it may be that older persons have had greater exposure to environmental triggers, thus allowing genetic susceptibilities to become more evident. Indeed, plasma markers of inflammation are under substantial genetic influence, even in older adults.[45] Therefore, confirmation of our findings in other large population studies of older adults with large numbers of fatal events will be important for clarifying the role of the *CRP* gene and risk of CVD.

Author Contributions: Dr L. Lange 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.
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Obtained funding: Kuller, Nickerson, Psaty, Tracy, Reiner.
Administrative, technical, or material support: Hindorff, Durda, Kuller, Nickerson, Psaty, Tracy, Reiner.
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Financial Disclosures: None reported.
Funding/Support: This study was supported by contracts N01-HC-85079 through N01-HC-85086, N01-HC-35129, and N01 HC-15103, and grant R01 HL-071862-03 (Dr Reiner) from the National Heart, Lung, and Blood Institute.
Role of the Sponsor: The funding organization participated in the design and conduct of CHS and monitored the study's data collection efforts and management. The funding organization did not participate in analysis and interpretation of the data for the manuscript but reviewed it for accuracy of the CHS study description and acknowledgment of study investigators and funding sources.
Additional Information: A full list of participating CHS investigators and institutions can be found at http://www.chs-nhlbi.org.
Acknowledgment: We thank Jill Perrotte, BS, and Kate M. Durda, Department of Pathology, University of Vermont College of Medicine, Burlington, for assistance with genotyping, and Jean Yee, BS, Department of Epidemiology, University of Washington, Seattle, for data management. All received support from the National Heart, Lung, and Blood Institute grant R01 HL-071862-03 for their contribution.

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The folly of mistaking a paradox for a discovery, a metaphor for a proof, a torrent of verbiage for a spring of capital truths, and oneself for an orator, is inborn in us.
—Paul Valéry (1871-1945)