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Original Contribution

Human Leukocyte Antigen Shared Epitope and Inflammation, Cardiovascular Disease, Cancer, and Mortality Among Postmenopausal Women in the Women's Health Initiative Rheumatoid Arthritis Study

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Specific alleles of the human leukocyte antigen (HLA)-DRB1 gene (*HLA-DRB1*) encode a "shared epitope" (SE) associated with rheumatoid arthritis (RA), especially more severe cyclic-citrullinated peptide antibody-positive (anti-CCP+) RA. We evaluated associations of number of SE alleles (0, 1, or 2) with total and cardiovascular disease (CVD) mortality and incident coronary heart disease (CHD), CVD, and cancer over a mean 8.9 (standard deviation, 3.5) years of follow-up, stratifying by baseline anti-CCP status (positive (+) vs. negative (−)). A longitudinal study, the Women's Health Initiative RA Study (1993–2010), sampled postmenopausal women who reported RA at baseline (1993–1998) or follow-up in the Women's Health Initiative, classified as anti-CCP+ RA ($n = 556$) or anti-CCP− non-RA ($n = 1{,}070$). Among anti-CCP+ RA women, SE alleles were not related to age-adjusted risks of CHD, CVD, or cancer or to total or CVD mortality. Among anti-CCP− non-RA women, age-adjusted hazard ratios for 1 and 2 SE alleles versus 0 SE alleles were 0.41 (95% confidence interval (CI): 0.34, 0.50) and 0.44 (95% CI: 0.27, 0.72), respectively, for CVD; 0.43 (95% CI: 0.37, 0.53) and 0.30 (95% CI: 0.16, 0.64), respectively, for CHD; and 0.62 (95% CI: 0.53, 0.73) and 0.52 (95% CI: 0.33, 0.83), respectively, for cancer. Associations persisted after adjustment for CVD risk factors, joint pain, rheumatoid factor positivity, and inflammatory markers (white blood cell count or cytokine level). In future studies, investigators should evaluate SE associations among anti-CCP− adults without RA and potential mechanisms.

cancer; cardiovascular disease; *HLA-DRB1* gene; human leukocyte antigen; mortality; rheumatoid arthritis; shared epitope

Abbreviations: ANA, antinuclear antibody; anti-CCP, antibodies to cyclic citrullinated peptides; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DMARD, disease-modifying antirheumatic drug; G-CSF, granulocyte colony-stimulating factor; HLA, human leukocyte antigen; IL, interleukin; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope; TNF-α, tumor necrosis factor α; WHI, Women's Health Initiative.

Rheumatoid arthritis (RA) is a systemic inflammatory disease associated with a 1.5- to 2-fold increased risk of total and cardiovascular disease (CVD) mortality and incident CVD (1–5), and possibly with solid-tumor cancer (6). CVD risk in persons with RA is related to diabetes, cigarette smoking, hypertension, hyperlipidemia, inflammation, joint pain, disability, and disease severity (3, 7). Specific alleles of the human leukocyte antigen (HLA)-DRB1 gene (*HLA-DRB1*) encode an amino acid sequence called the shared epitope (SE) that is strongly associated with development of RA (8, 9), particularly RA characterized by the presence of antibodies to cyclic citrullinated peptides (anti-CCP) (10). Smoking may strengthen the association of SE with anti-CCP positivity (11, 12). Anti-CCP-positive (anti-CCP+) RA also has stronger associations than anti-CCP-negative (anti-CCP−) RA with inflammation, RA severity, and greater risk of CVD

and mortality (13–19). Several research groups have reported associations between 1 or 2 SE alleles and higher risks of CVD, cancer, and mortality (11, 20, 21) and severity of RA (22). However, associations of SE with incident events and mortality have not been evaluated separately for more severe anti-CCP+ RA or for anti-CCP− individuals without RA.

In the current study, we evaluated associations of SE alleles with incident events and mortality separately for anti-CCP+ postmenopausal women (classified as anti-CCP+ RA) and anti-CCP− women with a low probability of having RA (classified as anti-CCP− non-RA). We hypothesized that for both groups, levels of inflammation, rates of incident CVD, coronary heart disease (CHD), and cancer, and rates of total and CVD mortality would be higher with 1 or 2 SE alleles than with 0 SE alleles.

METHODS

Participants and data collection in the Women's Health Initiative

The current report focuses on 1,626 postmenopausal women from the Women's Health Initiative (WHI) RA Study (WHI-RA), classified as anti-CCP+ RA ($n = 556$) or anti-CCP− non-RA ($n = 1,070$). As previously described, WHI-RA participants were sampled from the >10% of WHI participants who reported RA at baseline or during follow-up (23). The WHI (24) has previously been described in detail (23). Briefly, between 1993 and 1998, a total of 161,808 women aged 50–79 years (mean age = 62.8 years) were enrolled in clinical trials ($n = 68,132$) or an observational study ($n = 93,676$) at 40 clinical centers across the United States (24). Participants were asked whether they had arthritis and, if the answer was yes, whether it was RA. A chart review study indicated that only 14.7% of the 16,469 WHI participants who reported RA at baseline or at follow-up visits had clinical RA (i.e., positive predictive value = 14.7%). Therefore, the WHI-RA Study sampled 9,988 (65.8%) white, black, and Hispanic women who reported RA at baseline or follow-up with available serum samples ($n = 15,188$) for measurement of anti-CCP antibodies, rheumatoid factor (RF), and antinuclear antibodies (ANA) (23). Based on a second chart review study, WHI-RA participants were classified into one of 3 groups: 1) anti-CCP+ RA (positive predictive value = 80%; $n = 774$); 2) anti-CCP− (DMARD+) RA, defined by the use of disease-modifying antirheumatic drugs (DMARDs) (positive predictive value = 62%; $n = 649$); and 3) anti-CCP− non-RA, defined as women who reported RA but were anti-CCP− and did not report DMARD use, since they were unlikely to have RA (94% did not have clinical RA upon chart review) (25).

As previously described, HLA-DR typing and plasma cytokine levels were measured in a subset of the WHI-RA cohort (phase 2; $n = 2,993$) that included 1) 100% of anti-CCP+ women ($n = 774$, excluding 28 with insufficient plasma or DNA samples); 2) 100% of anti-CCP− women with self-reported DMARD use ($n = 649$); and 3) a 10% random sample of anti-CCP− women with no DMARD use who reported RA at baseline or at follow-up only ($n = 1,570$), plus all deaths among those women (23). SE alleles were successfully typed in 2,881 samples (112 were not measurable due to insufficient sample quality). Women who reported RA at follow-up only ($n = 1,072$) were excluded from our primary analyses to minimize misclassification bias, since chart review classifications relied on baseline anti-CCP+ and reported DMARD use. Effects of these exclusions on our results were evaluated in sensitivity analyses. Therefore, among the 1,809 WHI-RA participants who had reported RA at baseline in WHI, our primary analyses compared SE associations for anti-CCP+ women with RA ($n = 556$) and anti-CCP− women without RA ($n = 1,070$); results for anti-CCP−/DMARD+ RA are reported in the text as exploratory only, due to the small sample size ($n = 183$).

At the baseline WHI study visit, women reported their general health (excellent/very good, good, or fair/poor), age, educational attainment, ethnicity, and history of cigarette smoking, hypertension, diabetes, CVD, and cancer, and they were asked to rate the severity of joint pain (none, mild, moderate, or severe) and joint swelling during the prior 4 weeks (26). "High cholesterol" was defined as a self-reported high cholesterol level or use of lipid-lowering medication, since lipid concentrations were not measured in all WHI participants. White blood cell count was measured at baseline (27). Medication history, including use of DMARDs, was obtained by trained nurses' review of medication bottles at baseline and at follow-up visits. DMARDs included hydroxychloroquine, sulfasalazine, minocycline, methotrexate, leflunomide, azathioprine, cyclosporine, gold, cyclophosphamide, and antirheumatic biological agents (23, 25).

Laboratory methods

Anti-CCP, ANA, and RF were assayed in baseline serum samples in the Rheumatology Clinical Research Laboratory at the University of Colorado, as described previously (23). HLA-DR typing and cytokine levels were measured in the phase 2 sample ($n = 2,993$). Multiplex cytokine profiling was conducted at Stanford University using baseline plasma samples stored at −70°, as previously described (23). As previously described, HLA-DR typing was completed via polymerase chain reaction at the University of Pittsburgh (Dr. M. Trucco) using the current HLA sequence data (23). As in previous reports (23), the SE was defined by a 5-amino-acid sequence in the third hypervariable region of the first domain of the DR beta chain, consisting of 3 homologous amino acid sequence variants—QKRAA, QRRAA, and RRRAA—coded primarily by the *HLA-DRB1* alleles *0401, *0404, *0405, *0408, *0101, *0102, and *1001 (28).

Definitions of events

In this analysis, we evaluated adverse clinical events that occurred from study baseline (1993–1998) through 2010. Incident CHD was defined as adjudicated fatal and nonfatal myocardial infarction, angina, or coronary revascularization (angioplasty or coronary artery bypass surgery). Incident CVD was defined as incident CHD, stroke, transient ischemic attack, congestive heart failure, or carotid artery surgery. Events and deaths were identified through semiannual or annual follow-up telephone calls made by trained staff to family, friends, and medical care providers at each study site, review of obituaries, and the National Death Index; only 1%–2% of participants were lost to follow-up. Cardiovascular morbidity and cancer-related morbidity were centrally

adjudicated, as previously described (29). Underlying cause of death was used for classification of cause-specific mortality. Cardiovascular mortality included deaths from CHD, stroke, congestive heart failure, and other CVDs.

Statistical analysis

All analyses were performed separately for anti-CCP+ RA, anti-CCP− non-RA, and anti-CCP−/DMARD+ RA women, using SAS software, version 9.3 (SAS Institute, Inc., Cary, North Carolina). All models were 2-sided, with $\alpha = 0.05$. Differences in risk factors by number of SE alleles were tested with analysis of variance, χ^2 tests, or Kruskal-Wallis tests, as appropriate. Baseline CVD cases were excluded from analyses of incident CHD and CVD, and baseline cancer cases were excluded from analyses of incident cancer. Time to event was defined as the earlier of time from baseline to the date of the event or time from baseline to the end of follow-up. Due to the complex sampling design of our study, sampling weights, defined as 1/sampling fraction, were determined for each woman and used in the calculation of age-adjusted weighted incidence and mortality hazard ratios. Covariates and sensitivity analyses were identified from the literature and prior and current analyses of the WHI-RA cohort. Weighted age-adjusted incidence and mortality rates and 95% confidence intervals were calculated by number of SE alleles using direct methods, with the entire WHI cohort used as the standard population. Cox proportional hazards models were used to evaluate weighted age-adjusted associations between number of SE alleles and outcomes. The proportional hazards assumption was evaluated by including an interaction term for the interaction of SE alleles with time, and if the assumption was not met, an accelerated failure time model was used instead of a proportional hazards model. For outcomes significantly associated with a higher number of SE alleles, multivariable models were fitted, with further adjustment for diabetes, hypertension, high cholesterol, ever smoking, waist circumference, severe joint pain, RF positivity, ANA positivity, and log white blood cell count. Five sensitivity analyses were carried out: 1) We adjusted for DMARD use, as appropriate, or cytokine levels significantly associated with the SE; 2) we evaluated CHD without angina and CVD without angina or transient ischemic attack; 3) we restricted models to white women only, to evaluate potential confounding by race/ethnicity, given the small numbers of black and Hispanic women in the study; 4) we stratified by smoking status (never smokers vs. ever smokers), to evaluate confounding by smoking; and 5) we repeated analyses including anti-CCP−/DMARD+ RA in the anti-CCP− group and women who reported RA at follow-up only, to evaluate potential bias caused by those exclusions.

RESULTS

Participant characteristics and risk factors by number of SE alleles

The entire sample of 1,809 women included 556 anti-CCP+ RA subjects, 1,070 anti-CCP− non-RA subjects, and 183 anti-CCP− (DMARD+) RA subjects. For all groups, the distributions of SE alleles were similar by age group (≤60, 61–69, or >69 years; not shown). The prevalences of 1 and 2 SE alleles among anti-CCP+ RA women (49.1% and 17.6%, respectively) were much higher than those among anti-CCP− non-RA women (33.6% and 5.2%, respectively) (Table 1) or anti-CCP− (DMARD+) RA women (38.8% and 5.5%, respectively; not shown). Further analyses focused on the anti-CCP+ RA and anti-CCP− non-RA groups ($n = 1{,}626$) (Table 1), with results for the anti-CCP−/DMARD+ RA group being considered exploratory only, given the small sample size. The majority of participants were white (71.7%); 22.8% ($n = 371$) were African-American, and only 5.4% ($n = 93$) were Hispanic. Interestingly, among both anti-CCP+ RA women and anti-CCP− non-RA women, the prevalence of SE alleles differed by race/ethnicity (Table 1): There was a lower prevalence of 2 SE alleles among black women (5.6% and 1.8% for anti-CCP+ and anti-CCP−, respectively) than among white (22.3% and 6.2%, respectively) or Hispanic (16.2% and 5.8%, respectively) women. Among anti-CCP+ RA women, differences by number of SE alleles were statistically significant for mean body mass index (lower), ANA positivity (lower), and mean log white blood cell count (higher) (Table 1). Among anti-CCP− (non-RA) women, risk factors were similar by number of SE alleles (Table 1).

Among anti-CCP+ RA women, median concentrations of interleukin (IL)-1β, IL-2, IL-6, tumor necrosis factor α (TNF-α), interferon ɣ, and IL-10 were higher with a higher number of SE alleles ($P < 0.10$ for all) (Figure 1; also see Web Table 1, available at https://academic.oup.com/aje). Among anti-CCP− non-RA women, median concentrations of IL-10, IL-12, and granulocyte colony-stimulating factor (G-CSF) were higher with a higher number of SE alleles ($P < 0.05$ for all) (Figure 1, Web Table 2). Levels of other cytokines (IL-4, IL-5, IL-7, IL-8, IL-13, monocyte chemoattractant protein 1, macrophage inflammatory protein 1β, and IL-17) did not differ significantly by number of SE alleles among anti-CCP+ RA, anti-CCP− non-RA, and anti-CCP−/DMARD+ RA women (Web Tables 1–3). Finally, for both anti-CCP+ RA women and anti-CCP− non-RA women, associations of SE alleles with cytokines were similar by the presence or absence of RF (not shown).

SE, incident events, and mortality

Mean follow-up time was 8.9 (standard deviation, 3.5) years for anti-CCP+ RA and anti-CCP− non-RA women combined. The most common malignancies were lung cancer ($n = 48$), pancreatic cancer ($n = 14$), non-Hodgkin lymphoma ($n = 13$), and leukemia ($n = 10$), but cancer type was not related to number of SE alleles among anti-CCP+ RA, anti-CCP− non-RA, or anti-CCP−/DMARD+ RA women (not shown). Among anti-CCP+ RA women, weighted age-adjusted rates (Table 2) and weighted age-adjusted hazard ratios (Table 3) for total and CVD mortality and incident CVD, CHD, and cancer were not significantly different for 1 or 2 SE alleles versus 0 SE alleles. Results were similar in models 1) adjusted for DMARD use, 2) restricted to white women only, 3) additionally including anti-CCP+ women who reported RA at follow-up only, and 4) for CHD without angina and CVD without angina or transient ischemic attack (not shown). Finally, among anti-CCP+ RA women, the presence of specific alleles (*HLA-DRB1**0401, *0404, or *04

Table 1. Baseline Characteristics of Participants With Rheumatoid Arthritis According to Number of Human Leukocyte Antigen (HLA)-DRB1 Gene (*HLA-DRB1*) Shared Epitope Alleles and Anti-CCP Status ($n = 1,626$), Women's Health Initiative Rheumatoid Arthritis Study, 1993–2010[a]

Characteristic	Anti-CCP Status and No. of Shared Epitope Alleles												
	Anti-CCP+ RA ($n = 556$)							Anti-CCP− Non-RA ($n = 1,070$)					
	0 ($n = 185$)		1 ($n = 273$)		2 ($n = 98$)		*P* Value[b]	0 ($n = 655$)		1 ($n = 359$)		2 ($n = 56$)		*P* Value[b]
	%	Mean	%	Mean	%	Mean		%	Mean	%	Mean	%	Mean	
Total	33.3		49.1		17.6			61.2		33.6		5.2		
Age, years		64.1		64.0		63.3	0.66		66.6		66.3		66.4	0.84
Race/ethnicity							<0.0001							<0.0001
White	54.1		70.7		85.7			69.2		80.0		87.5		
Black	37.8		23.4		8.2			26.1		15.0		7.1		
Hispanic	8.1		5.9		6.1			4.7		5.0		5.4		
Body mass index[c]		29.0		27.7		27.2	0.02		30.1		29.5		28.8	0.19
Waist circumference, cm		88.0		86.6		86.7	0.51		91.7		91.3		88.0	0.21
Log WBC count, per 10^9 cells/L		1.77		1.84		1.86	0.03		1.80		1.83		1.81	0.25
Prevalent CHD	6.0		10.3		9.2		0.27	20.0		20.1		12.5		0.39
Prevalent CVD	8.7		12.1		10.2		0.50	24.7		25.1		19.6		0.67
Prevalent cancer	13.5		12.5		10.2		0.71	15.7		18.1		17.9		0.62
Severe joint pain	25.4		20.5		28.6		0.17	21.8		22.8		14.3		0.34
General health[d]														
Excellent/very good	28.1		26.7		15.3		0.13	30.8		31.5		35.7		0.92
Good	44.9		43.5		55.1			39.8		40.4		41.1		
Fair/poor	25.4		28.2		28.6			28.5		27.9		23.2		
Smoking[d]														
Never smoker	42.7		37.0		42.9		0.70	50.2		48.5		46.4		0.73
Former smoker	47.0		50.2		43.9			37.4		39.6		46.4		
Current smoker	10.3		11.7		9.2			9.6		10.6		7.1		
Hypertension	35.7		41.4		28.6		0.06	49.9		48.5		42.9		0.48
Diabetes	9.2		5.9		3.1		0.12	14.5		15.6		10.7		0.62
High cholesterol[e]	14.6		9.9		9.2		0.25	19.8		21.2		14.3		0.53
Rheumatoid factor-positive	82.2		85.3		91.8		0.09	13.1		14.5		7.1		0.31
ANA-positive (≥1:320 titer)	20.5		12.1		12.2		0.03	10.4		10.6		5.4		0.47

Abbreviations: ANA, antinuclear antibody; anti-CCP, antibodies to cyclic citrullinated peptides; CHD, coronary heart disease; CVD, cardiovascular disease; RA, rheumatoid arthritis; WBC, white blood cell.

[a] Women who reported RA at a follow-up visit only were excluded.

[b] *P* value from 1-way analysis of variance or a χ^2 test for differences by number of shared epitopes.

[c] Weight (kg)/height $(m)^2$.

[d] Column percentages do not sum to 100 because of missing data.

[e] "High cholesterol" was defined as a self-reported high cholesterol level or use of lipid-lowering medication, since lipid concentrations were not measured in all participants.

alleles) versus 0 SE alleles was also not significantly associated with higher risk of events or mortality (not shown).

In contrast, among anti-CCP− non-RA women, weighted age-adjusted incidence rates (Table 2) and weighted age-adjusted hazard ratios (Table 3) for CVD, CHD, and cancer were ≥40% lower for women with 1 or 2 SE alleles versus 0 SE alleles. In multivariable models, age-adjusted hazard ratios were essentially unchanged after additional adjustment for smoking, diabetes, hypertension, high cholesterol, waist circumference, severe joint pain, RF positivity, ANA positivity, and log white blood cell count (Table 4), after additional adjustment for potentially protective cytokines (IL-10, IL-12, and G-CSF), or after replacing waist circumference with body mass index (not shown). Furthermore, for anti-CCP− non-RA women, associations between a higher number of SE alleles and lower risks of CVD, CHD, and cancer persisted after 1) restriction to white women only, 2) restriction to never smokers only or to ever (current and former) smokers only, 3) inclusion of women with anti-CCP−/DMARD+ RA, before and after adjustment for DMARD use, and 4) evaluation of CHD without

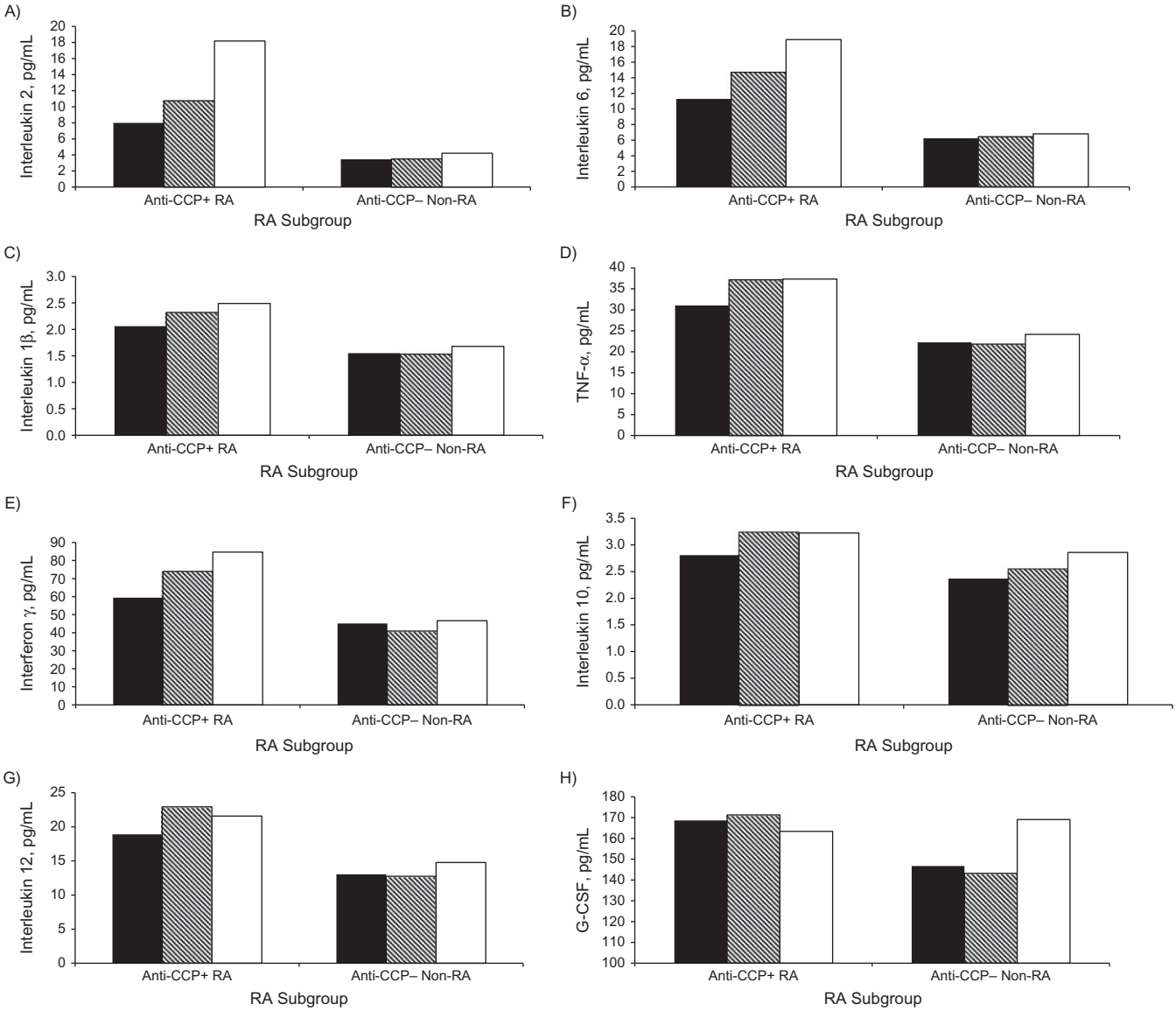

Figure 1. Median cytokine concentrations (pg/mL) according to the presence of antibodies to cyclic citrullinated peptides (anti-CCP) and number of human leukocyte antigen (HLA)-DRB1 gene (*HLA-DRB1*) shared epitope (SE) alleles among participants reporting rheumatoid arthritis (RA), Women's Health Initiative Rheumatoid Arthritis Study, 1993–2010. Women reporting RA at a follow-up visit only were excluded. Black columns, 0 SE alleles; striped columns, 1 SE allele; white columns, 2 SE alleles. *P* values presented below are from Kruskal-Wallis tests of the difference in median cytokine levels by number of SE alleles. A) Interleukin 2 (anti-CCP-positive (anti-CCP+) RA participants: $P = 0.02$; anti-CCP-negative (anti-CCP−) non-RA participants: $P = 0.16$); B) interleukin 6 (anti-CCP+ RA: $P = 0.001$; anti-CCP− non-RA: $P = 0.24$); C) interleukin 1β (anti-CCP + RA: $P = 0.04$; anti-CCP− non-RA: $P = 0.31$); D) tumor necrosis factor α (TNF-α) (anti-CCP+ RA: $P = 0.03$; anti-CCP− non-RA: $P = 0.22$); E) interferon γ (anti-CCP+ RA: $P = 0.01$; anti-CCP− non-RA: $P = 0.13$); F) interleukin 10 (anti-CCP+ RA: $P = 0.07$; anti-CCP− non-RA: $P = 0.003$); G) interleukin 12 (anti-CCP+ RA: $P = 0.19$; anti-CCP− non-RA: $P = 0.03$); H) granulocyte colony-stimulating factor (G-CSF) (anti-CCP+ RA: $P = 0.85$; anti-CCP− non-RA: $P = 0.04$).

angina and CVD without angina or transient ischemic attack (not shown). Results for cancer, CHD, and CVD were similar in sensitivity analyses including anti-CCP− women who reported RA at follow-up, except that for CHD and CVD, protective associations with 2 SE alleles were attenuated (not shown).

Finally, in exploratory analyses carried out among 183 anti-CCP−/DMARD+ RA women, mean body mass index was lower and the prevalence of cancer was higher (5%, 15.5%, and 30%) for 0, 1, and 2 alleles, respectively ($P < 0.05$ for both; not shown). The number of SE alleles was not related to total or CVD mortality or to incident CVD or CHD. However, for incident malignancies, the weighted hazard ratio for 1 or 2 SE alleles versus 0 SE alleles was 0.44 (95% confidence interval: 0.18, 1.06; $P = 0.067$) after adjustment for age, waist circumference,

Table 2. Weighted Age-Adjusted Incidence Rates of Total and Cardiovascular Disease Mortality and Incident Cardiovascular Disease, Coronary Heart Disease, and Cancer Among Women With Rheumatoid Arthritis, According to Number of Human Leukocyte Antigen (HLA)-DRB1 Gene (*HLA-DRB1*) Shared Epitope Alleles and Anti-CCP Status ($n = 1,626$), Women's Health Initiative Rheumatoid Arthritis Study, 1993–2010[a]

Outcome and No. of SE Alleles	Anti-CCP Status							
	Anti-CCP+ RA ($n = 556$)				Anti-CCP− Non-RA ($n = 1,070$)			
	No. of Events	Total No. of Women	IR	95% CI	No. of Events	Total No. of Women	IR	95% CI
Total mortality								
0	36	214	16.11	9.56, 28.02	497	3,391	12.46	10.69, 14.55
1	74	346	20.50	13.99, 30.38	281	2,161	10.67	8.73, 13.08
2	25	125	18.54	9.50, 36.53	38	265	19.21	12.23, 30.98
CVD mortality[b]								
0	10	196	4.64	1.85, 14.13	61	2,817	1.89	1.29, 2.90
1	7	305	2.25	0.82, 6.37	43	1,886	1.89	1.14, 3.19
2	2	113	1.57	0.34, 7.31	5	235	3.10	1.10, 9.46
Incident CVD[b]								
0	25	196	13.20	6.99, 25.50	426	2,817	14.49	12.29, 17.10
1	41	305	13.47	7.97, 22.91	129	1,886	6.01	4.52, 8.04
2	15	113	13.48	5.75, 31.84	16	235	6.89	3.32, 14.60
Incident CHD[b,c]								
0	16	196	8.30	3.77, 19.05	228	2,817	7.47	5.96, 9.38
1	21	305	6.50	3.09, 13.81	79	1,886	3.61	2.58, 5.18
2	6	113	5.29	1.46, 20.68	5	235	3.18	1.13, 9.66
Cancer[d]								
0	21	182	13.20	6.74, 27.27	494	3,037	16.68	14.29, 19.49
1	37	307	13.17	7.59, 23.02	198	1,889	10.44	8.20, 13.29
2	17	110	16.42	7.22, 38.20	20	222	10.56	5.52, 20.32

Abbreviations: anti-CCP, antibodies to cyclic citrullinated peptides; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; IR, incidence rate; RA, rheumatoid arthritis.

[a] Women who reported RA at a follow-up visit only were excluded.

[b] Analyses of CVD mortality and incident CVD and CHD excluded women with prevalent CVD at baseline.

[c] IRs are from an accelerated failure time model.

[d] Analyses of incident cancer excluded women with prevalent cancer at baseline.

smoking, diabetes, hypertension, high cholesterol, severe joint pain, RF positivity, ANA positivity, and log white blood cell count, with prevalent cancer cases excluded. Results were similar when prevalent cancer cases were included (not shown). Finally, among anti-CCP−/DMARD+ women, median levels of IL-6, IL-1β, TNF-α, IL-10, and IL-12 were modestly higher with a higher number of SE alleles, although not statistically significantly (Web Table 3).

DISCUSSION

The current study provided a rare opportunity to evaluate associations of number of SE alleles with total and CVD mortality and incident CVD, CHD, and cancer among postmenopausal women with RA defined by anti-CCP positivity (anti-CCP+ RA), as well as a large sample of anti-CCP− postmenopausal women who reported arthritis and RA but were unlikely to have clinical RA. Consistent with prior studies (10, 30), SE alleles were strongly associated with anti-CCP+ RA, but in that group, SE alleles were not associated with higher total or CVD mortality or incidence of CVD, CHD, or cancer, despite associations with higher levels of inflammatory cytokines (IL-2, IL-6, IL-1β, TNF-α, and interferon γ). In contrast, among anti-CCP− women without RA (non-RA), the 38.2% with 1 or 2 SE alleles had an approximately 50% lower risk of incident CVD, CHD, or cancer than women with 0 SE alleles. Among anti-CCP− non-RA women, SE alleles were also associated with higher levels of the potentially protective cytokines IL-10, IL-12, and G-CSF. The protective associations of SE alleles with CVD, CHD, and cancer among anti-CCP− women without RA persisted after adjustment for a wide range of confounders, including these potentially protective cytokines, and in a wide range of sensitivity analyses.

Among anti-CCP+ RA women in our study, SE alleles were not significantly associated with higher risk of total or

Table 3. Weighted Age-Adjusted Hazard Ratios for Total and Cardiovascular Disease Mortality and Incident Cardiovascular Disease, Coronary Heart Disease, and Cancer Among Women With Rheumatoid Arthritis, According to Number of Human Leukocyte Antigen (HLA)-DRB1 Gene (*HLA-DRB1*) Shared Epitope Alleles and Anti-CCP Status ($n = 1,626$), Women's Health Initiative Rheumatoid Arthritis Study, 1993–2010[a]

Outcome and No. of SE Alleles	Anti-CCP Status			
	Anti-CCP+ RA ($n = 556$)		Anti-CCP− Non-RA ($n = 1,070$)	
	HR	95% CI	HR	95% CI
Total mortality				
0	1.0	Referent	1.0	Referent
1	1.27	0.85, 1.90	0.90	0.78, 1.04
2	1.24	0.74, 2.06	1.17	0.94, 1.62
CVD mortality[b]				
0	1.0	Referent	1.0	Referent
1	0.46	0.17, 1.24	1.03	0.70, 1.52
2	0.31	0.06, 1.65	1.12	0.45, 2.79
Incident CVD[b]				
0	1.0	Referent	1.0	Referent
1	1.04	0.63, 1.71	0.41	0.34, 0.50
2	1.07	0.56, 2.02	0.44	0.27, 0.72
Incident CHD[b,c]				
0	1.0	Referent	1.0	Referent
1	0.83	0.43, 1.59	0.43	0.37, 0.53
2	0.66	0.26, 1.68	0.30	0.16, 0.64
Cancer[d]				
0	1.0	Referent	1.0	Referent
1	1.00	0.59, 1.70	0.62	0.53, 0.73
2	1.31	0.69, 2.48	0.52	0.33, 0.83

Abbreviations: anti-CCP, anti-cyclic citrullinated peptides; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; RA, rheumatoid arthritis.

[a] Women who reported RA at a follow-up visit only were excluded.

[b] Analyses of CVD mortality and incident CVD and CHD excluded women with prevalent CVD at baseline.

[c] HRs are from an accelerated failure time model.

[d] Analyses of incident cancer excluded women with prevalent cancer at baseline.

CVD mortality or incident CVD, CHD, or cancer, even when analysis was restricted to *HLA-DRB1**0401, *0404, or *04 alleles versus 0 SE alleles. This differs from prior studies that did not stratify by anti-CCP+ status (11, 20, 21). Among 178 RA patients, CVD mortality and CVD incidence were not associated with the SE but were associated with *HLA-DRB1**0404 (20). In the Early Rheumatoid Arthritis Study ($n = 767$), ischemic heart disease mortality (hazard ratio = 2.02, 95% confidence interval: 1.04, 3.94) and cancer mortality (hazard ratio = 2.18, 95% CI: 1.17, 4.08) over 18 years were higher for 2 SE alleles versus 0 or 1 SE alleles (21). Finally, the Norfolk Arthritis Register cohort included 1,022 patients with inflammatory polyarthritis, and 472 (46%) had RA at baseline, of whom 60% were anti-CCP+. In the Norfolk Arthritis Register cohort, overall hazard ratios for 2 SE alleles versus 0 SE alleles were 1.58 for total mortality and 1.68 for CVD mortality, with the strongest association being seen for *HLA-DRB1**01 and *04 alleles (11). Although the independent effect of anti-CCP+ was not evaluated, the hazard ratio for CVD death was 7.81 (95% CI: 2.63, 23.22) for anti-CCP+ current smokers with 2 SE alleles as compared with anti-CCP− never smokers with 0 or 1 allele (11). These results agree with our previous report from the WHI RA Study, showing that mortality rates were approximately doubled for women with anti-CCP+ RA compared with no RA and for current smokers versus never smokers (15). These results suggest that studies of SE alleles in RA should stratify by anti-CCP+ status.

Our study also found novel and contrasting associations of SE alleles with cytokines for anti-CCP− non-RA women versus anti-CCP+ RA women. Among anti-CCP− non-RA women, higher numbers of SE alleles were associated with higher levels of potentially antiinflammatory IL-10 (31), antiangiogenic IL-12 (32), and G-CSF (33), which have been evaluated as possible therapeutic targets for RA (34, 35). In contrast, among anti-CCP+ RA women, higher numbers of SE alleles were associated with higher levels of proinflammatory cytokines: IL-1β, IL-2, IL-6, TNF-α, and interferon ɣ. These cytokines are associated with RA pathogenesis (36, 37) and may contribute to the more severe, erosive RA that has been associated with anti-CCP+ in multiple studies (17, 18). Associations were similar when results were stratified by the presence or absence of RF, although cytokine levels were highest for women who were both anti-CCP+ and RF+, as previously reported (23, 38). These cytokine associations suggest a mechanism for prior studies that associated RA severity with anti-CCP+ and RF+ status rather than the SE (19), that associated RA severity with both anti-CCP+ status and SE alleles (39), or which suggested that the association of the SE with RA severity was partially mediated by anti-CCP+ status (40). Mechanisms that explain contrasting associations of the SE with outcomes and cytokines for anti-CCP+ RA status versus anti-CCP− non-RA status are speculative. The SE alleles are located in a peptide-binding groove and are believed to influence antigenic binding to the HLA molecule, as well as antigen presentation to T lymphocytes (41). Therefore, in RA, the SE may potentiate synovitis by facilitating binding of arthritogenic peptides to T cells (41, 42). In contrast, among non-RA (anti-CCP−) individuals, the SE may enhance immune surveillance that leads to early identification and destruction of malignant cells by facilitating antigen presentation of tumorigenic peptides. Alterations or defects in HLA have also been implicated in the pathogenesis of multiple solid organ cancers (43). The HLA also plays an important role in the recognition and destruction of malignant cells by T lymphocytes, and enhancement of HLA expression has been targeted as a strategy in cancer immunotherapy (43).

Our study had several strengths and limitations. First, it was conducted within a large, ethnically diverse group of postmenopausal women, the WHI study population, with standardized data collection and long-term follow-up. Although numbers were too small to evaluate associations among black or Hispanic women only, results were similar when analyses were

Table 4. Weighted Multivariable-Adjusted Hazard Ratios for Incident Cardiovascular Disease, Coronary Heart Disease, and Cancer Among Anti-CCP− Non-RA[a] Participants ($n = 1,070$), Women's Health Initiative Rheumatoid Arthritis Study, 1993–2010[b]

Baseline Risk Factor[c]	CVD[d]		CHD[d,e]		Cancer[f]	
	HR	95% CI	HR	95% CI	HR	95% CI
No. of *HLA-DRB1* shared epitope alleles						
1 (vs. 0)	0.33	0.26, 0.41	0.30	0.25, 0.37	0.62	0.53, 0.74
2 (vs. 0)	0.45	0.27, 0.74	0.27	0.15, 0.58	0.44	0.28, 0.69
Age, years	1.04	1.03, 1.05	1.07	1.05, 1.10	1.04	1.03, 1.05
Ever smoking	1.40	1.18, 1.67	2.27	1.66, 3.30	0.94	0.81, 1.10
Diabetes	1.74	1.29, 2.34	3.55	2.29, 5.98	2.44	1.91, 3.11
Hypertension	1.55	1.29, 1.86	1.49	1.14, 2.05	0.64	0.54, 0.76
High cholesterol[g]	1.37	1.10, 1.70	2.76	1.96, 4.17	1.08	0.88, 1.32
Waist circumference, cm	1.00	0.99, 1.01	0.99	0.98, 1.00	1.00	1.00, 1.01
Severe joint pain (vs. none, mild, or moderate)	1.35	1.10, 1.67	2.02	1.49, 2.94	0.46	0.36, 0.60
Rheumatoid factor-positive	1.62	1.27, 2.06	1.62	1.17, 2.42	0.37	0.26, 0.52
ANA-positive (≥1:320 titer)	0.69	0.48, 0.99	0.93	0.63, 1.50	0.92	0.68, 1.24
Log WBC count, per 10^9 cells/L	2.91	2.19, 3.87	2.50	1.56, 4.43	1.14	0.85, 1.53

Abbreviations: ANA, antinuclear antibody; anti-CCP, anti-cyclic citrullinated peptides; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HLA, human leukocyte antigen; *HLA-DRB1*, HLA-DRB1 gene; HR, hazard ratio; RA, rheumatoid arthritis; WBC, white blood cell.

[a] Anti-CCP-negative women with a low probability of having RA.

[b] Women who reported RA at a follow-up visit only were excluded.

[c] Categorical variables were analyzed dichotomously (yes/no) unless otherwise indicated.

[d] Analyses of incident CHD and CVD excluded women with prevalent CVD at baseline, leaving 766 women for analysis.

[e] HRs are from an accelerated failure time model.

[f] Analyses of incident cancer excluded women with prevalent cancer at baseline, leaving 837 women for analysis.

[g] "High cholesterol" was defined as a self-reported high cholesterol level or use of lipid-lowering medication, since lipid concentrations were not measured in all participants.

restricted to white women only (72% of the sample). Furthermore, HLA SE alleles are associated with RA risk primarily among Caucasians, and other HLA alleles may be important in Japanese, Chinese, and Native American populations. A potential limitation of the study is that all participants were sampled from the >10% of WHI participants who reported RA at baseline or follow-up. However, chart review studies suggested that more than 85% of these women did not have clinical RA and reclassified them into anti-CCP+ RA, anti-CCP− RA (based on DMARD use), and anti-CCP− non-RA groups (15, 23, 25, 44). This provided us with the rare opportunity to evaluate associations of SE alleles with outcomes separately among large samples of anti-CCP+ RA women ($n = 556$) and anti-CCP− women who were unlikely to have RA ($n = 1,070$). The comparison of anti-CCP+ status with anti-CCP− status is unlikely to have been affected by potential misclassification of RA. Correspondingly, results for anti-CCP− (DMARD−) non-RA women were similar if anti-CCP−/DMARD+ women were included, before or after adjustment for DMARD use. In addition, results were similar when women who reported RA at follow-up visits only were included, except that for anti-CCP− non-RA women, associations of 2 SE alleles (vs. 0 SE alleles) with CHD and CVD were attenuated. This suggests that protective associations of SE alleles with incident CHD and CVD (but not cancer) may be restricted to women who never become anti-CCP+ or develop RA. Finally, our results may have been affected by survival bias, if women with 1 or 2 SE alleles died prior to the start of WHI or were too ill to enroll in WHI. However, although mean age in this study (64 years for anti-CCP+ women and 66 years for anti-CCP− women) was higher than in prior studies (mean or median, 54–60 years) (11, 20, 21), the frequencies of 1 or 2 SE alleles were similar for older women (ages >69 years) and younger women (ages ≤60 years). Additional studies are needed to determine whether these results are generalizable to other groups, including anti-CCP− RA women, postmenopausal women who never reported RA, younger women, men, and persons with a race/ethnicity other than non-Hispanic white.

In conclusion, among women with anti-CCP+ RA, SE alleles were associated with higher cytokine levels but not with adverse CVD outcomes, coronary heart disease, or cancer. Our results suggest that associations of SE alleles with adverse clinical outcomes are likely to be partially confounded by strong associations of SE alleles with anti-CCP+ RA, and future studies should evaluate this group separately. Our novel findings that among anti-CCP− women without RA, the presence of 1 or 2 SE alleles was associated with approximately 50% lower risks of CVD, CHD, and cancer, as well as with higher levels of

possibly protective cytokines (IL-10, IL-12, and G-CSF), require further study and replication.

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