Genes and Immunity (2015) **16,** 446–451

www.nature.com/gene

ORIGINAL ARTICLE

The role of genetic variants in *CRP* in radiographic severity in African Americans with early and established rheumatoid arthritis

MI Danila[1], AO Westfall[2], K Raman[1], L Chen[1], RJ Reynolds[1], LB Hughes[1], DK Arnett[2], G McGwin[2], AJ Szalai[1], DM van der Heijde[3], D Conn[4], LF Callahan[5], LW Moreland[6] and SL Bridges Jr[1]

This study investigates the association of *CRP* (C-reactive protein) single-nucleotide polymorphisms (SNPs) with plasma CRP levels and radiographic severity in African Americans with early and established rheumatoid arthritis (RA). Using a cross-sectional case-only design, *CRP* SNPs were genotyped in two independent sets of African Americans with RA: Consortium for the Longitudinal Evaluation of African Americans with RA (CLEAR 1) and CLEAR 2. Radiographic data and CRP measurements were available for 294 individuals from CLEAR 1 (median (interquartile range (IQR) 25–75) disease duration of 1 (0.6–1.6) year) and in 407 persons from CLEAR 2 (median (IQR 25–75) disease duration of 8.9 (3.5–17.7) years). In CLEAR 1, in adjusted models, the minor allele of rs2808630 was associated with total radiographic score (incident rate ratio 0.37 (95% confidence interval (CI) 0.19–0.74), *P*-value = 0.0051). In CLEAR 2, the minor allele of rs3093062 was associated with increased plasma CRP levels (*P*-value = 0.002). For each rs3093062 minor allele, the plasma CRP increased by 1.51 (95% CI 1.15–1.95) mg dl^{-1} when all the other covariates remained constant. These findings have important implications for assessment of the risk of joint damage in African Americans with RA.

Genes and Immunity (2015) **16,** 446–451; doi:10.1038/gene.2015.24; published online 30 July 2015

INTRODUCTION

Rheumatoid arthritis (RA), a common form of inflammatory arthritis affecting synovial joints, has a variable clinical expression ranging from mild disease to severe joint destruction.[1] Although the biological processes underlying the pathophysiology of RA are not completely understood, systemic inflammation, as reflected by serum C-reactive protein (CRP) levels, is thought to represent a key component. CRP is found in the synovial fluid of RA patients[2] and can bind to lymphocytes, monocytes and other inflammatory cells.[3–5] Serum CRP level is commonly used to assess disease activity in RA patients[6] and is part of the Disease Activity Score in 28 joints (DAS28-CRP).[7] Serum CRP levels are also included in the American College of Rheumatology treatment response criteria.[8] The importance of serum CRP in RA is also highlighted by the finding that levels of this acute phase reactant influences physicians' decisions in changing treatment in RA.[9]

Single-nucleotide polymorphisms (SNPs) in *CRP* have been shown to be associated with serum CRP levels[10–13] and their biological role has been evaluated in different disease states including RA, cardiovascular disease, Alzheimer's disease, colorectal cancer and chronic kidney disease.[10,14–17] For instance, among African Americans without known cardiovascular disease, the minor allele of the *CRP* SNP rs3093058 is associated with higher serum CRP levels, while the minor allele of rs1205 is associated with lower CRP serum levels.[12]

There is great variability in the minor allele frequency (MAF) of *CRP* SNPs among different ethnic groups.[10,18] *CRP* SNP rs3093058 is part of a haplotype associated with incident stroke in African Americans,[12] but this SNP is monomorphic (MAF = 0) in European Americans and thus does not contribute to the risk of stroke in that ethnic group.[12] Furthermore, the MAF of another haplotype-tagging SNP in *CRP* (rs3093066) is 0.23 in African Americans, but this locus is monomorphic in persons of European ancestry.[18] Given the relevance of CRP to RA pathogenesis and clinical decision making, such differences in genotype distributions could have important implications for pathogenesis in patients with RA.

CRP is indisputably a component of the inflammatory process in RA, and plasma CRP levels are associated with radiographic damage among RA patients.[19] However, the relationship between radiographic damage in RA and genetic variants within *CRP* has not explicitly been investigated, particularly in understudied ethnic minority populations. In the current study, we measured plasma CRP levels and genotyped 11 polymorphic SNPs in *CRP* in a sample of well-characterized African Americans with RA with early disease (CLEAR 1) and with predominantly long-standing disease (CLEAR 2). Thus, these analyses provide insight into the role of CRP at different phases of the disease. Specifically, we tested whether *CRP* polymorphisms were associated with radiographic severity and with plasma CRP levels in early and long-standing disease to evaluate whether systemic inflammation and RA-induced joint damage have common genetic determinants.

[1]Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA; [2]Department of Biostatistics, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA; [3]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Medicine, Emory University, Atlanta, GA, USA; [5]Department of Medicine, University of North Carolina, Chapel Hill, NC, USA and [6]Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. Correspondence: Dr MI Danila, Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, 1720 Second Avenue, South, FOT 858, Birmingham 35294-3408, AL, USA.
E-mail: mdanila@uab.edu
Received 24 March 2015; revised 22 May 2015; accepted 29 May 2015; published online 30 July 2015

RESULTS

The baseline characteristics of the study samples are presented in Table 1. More detailed information about the clinical characteristics of the CLEAR participants has been previously published.[20] Table 1 shows that compared with African Americans with RA from CLEAR 1 subset, the participants enrolled in CLEAR 2 had more radiographic damage, were older, had a longer disease duration, a lower median tender joint count and were more likely to be autoantibody positive (rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACPA)). In addition, there were differences in use of disease-modifying anti-rheumatic drugs (DMARDs) and biological agents. Because enrollment in CLEAR 1 occurred between 2000 and 2005, a relatively low percentage (~4%) of participants used biological agents (etanercept, infliximab and anakinra). Enrollment in CLEAR 2 occurred between 2006 and 2011, so a higher percentage (~36%) of participants had been on biological agents (etanercept, infliximab, anakinra, adalimumab, abatacept or rituximab). Disease activity score (DAS28) could not be calculated in the CLEAR 1 participants, since global health scores by visual analog scale were not available. However we were able to calculate DAS28-3(CRP) using total joint count, swollen joint count and CRP level using the formula DAS28-3(CRP) = (0.56 × SQRT(TJC28)+0.28 × SQRT (SJC28)+0.36 × ln(CRP+1)) × 1.10+1.15, which we have reported in detail previously.[21] The Pearson correlation between the CRP SNPs and DAS28-3(CRP) ranged from − 0.09 to 0.09. Since global health scores by visual analog scale were available for CLEAR 2 participants, we were able to calculate DAS28(CRP) with four variables. The Pearson correlation between CRP SNPs and DAS28 (CRP) ranged from –0.12 to 0.07. Thus, as expected, there was no demonstrable association between DAS28 scores (which vary widely during the patient's course, depending on flare/remission status) and CRP genotype.

Associations of *CRP* polymorphisms with total radiographic scores

The median (interquartile range 25–75) modified total Sharp score (mTSS) at enrollment in CLEAR 1 was 0 (0–2), while in CLEAR 2, the median (interquartile range 25–75) mTSS was 6 (0–31), as previously reported.[20] As noted above, mTSS of 0 was noted in 200 (68%) CLEAR 1 participants and 248 (61%) CLEAR 2 participants. The results of the univariate and multivariate analyses for the associations of *CRP* genotypes with mTSS are shown in Tables 2 and 3. In the multivariate analysis of CLEAR 1, the minor allele of *CRP* variant rs2808630 was associated with lower total radiographic score after adjusting for RA disease duration, age, gender, autoantibody status (RF and ACPA), CRP level and use of traditional or biological DMARD (incident rate ratio 0.37 (95% confidence interval (CI) 0.19–0.74), *P*-value = 0.0051) (Table 2). For each C-allele of the rs2808630, the mTSS decreased by 73% (95% CI 26–81%) when all the other covariates remained constant. Because ancestry could potentially confound the association between rs2808630 and the total radiographic score, we tested the mean European admixture proportion across genotypes for rs2808630. The proportion of European admixture was available for 69% of the CLEAR 1 participants included in the analysis. The mean (s.d.) European admixture proportion was 0.18 (0.05), 0.19 (0.12), 0.16 (0.1) for rs2808630 CC, CT and TT, respectively. There was no statistically significant difference in the proportion of European admixture across the genotypes ($P = 0.26$), which demonstrates that the proportion of European admixture does not confound the association between rs2808630 and total radiographic score. In fact, after adjustment for the proportion of European admixture, there was an association between the minor allele of CRP variant rs2808630 and lower total radiographic score (incident rate ratio 0.43 (95% CI 0.19–0.97), *P*-value = 0.0432) (Table 2). None of the CRP SNPs was associated with radiographic severity in CLEAR 2 after adjusting for relevant covariates (Table 3).

Table 1. Characteristics of African American participants with RA from CLEAR registry

Variable	*CLEAR 1* (N = *294*)	*CLEAR 2* (N = *407*)
Age, years, mean (s.d.)	50.7 (13.4)	56.4 (11.8)
Age at diagnosis, years, mean (s.d.)	49.6 (13.4)	44.9 (12.7)
BMI, kg m^{-2}, mean (s.d.)[a]	31.3 (7.6)	31.9 (7.7)
Disease duration, years, median (IQR 25–75)	1 (0.6–1.6)	8.9 (3.5–17.7)
Swollen joint count, median (IQR 25–75)	4 (1–9)	5 (1–13)
Tender joint count, median (IQR 25–75)	7 (2–18)	6 (2–14)
Gender, female, *N* (%)	242 (82.3)	349 (85.8)
Smoking, ever, *N* (%)[b]	156 (53.2)	210 (51.6)
RF status, positive, *N* (%)[c]	204 (70.3)	328 (80.8)
ACPA status, positive, *N* (%)[d]	183 (63.1)	292 (71.9)
Shared epitope status, present, *N* (%)[e]	122 (41.5)	154 (37.9)
Corticosteroids, ever, *N* (%)[f]	154 (79.8)	379 (93.8)
Synthetic DMARD use, ever, *N* (%)[g]	247 (84)	388 (98.5)
Biologic DMARD use, ever, *N* (%)[h,i]	12 (3.9)	145 (35.9)
mTSS, median (IQR 25–75)	0 (0–2)	6 (0–31)
Percent European admixture, mean (s.d.)[j]	17(10.6)	15.4 (9.3)
CRP, mg dl^{-1}, median (IQR 25–75)[k]	5.1 (1.8–9.4)	4.2 (1.5–8.9)

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; CLEAR, Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; IQR, interquartile range; mTSS, modified total Sharp score; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope. [a]Missing for 17 persons. [b]Missing for 1 person. [c]Missing for 5 persons. [d]Missing for 5 persons. [e]Missing for 1 persons. [f]Missing for 104 persons. [g]Missing for 2 persons. [h]Missing for 3 persons. [i]Biologic DMARDs: etanercept, infliximab, adalimumab and anakinra. [j]Missing for 159 persons. [k]Missing for 5 persons.

Associations of *CRP* polymorphisms with plasma CRP levels

The results of the univariate and multivariate analyses for the associations of *CRP* polymorphisms with baseline plasma CRP are shown in Tables 4 and 5. After adjusting for age, gender, BMI, number of swollen and tender joints, autoantibody status and corticosteroid use, *CRP* genotype rs3093062 was associated with increased plasma CRP levels in CLEAR 2 (mean estimate (95% CI) 0.18 (0.06–0.29), $P = 0.0021$) (Table 5). Thus, for each rs3093062 A minor allele, the plasma CRP increased by 1.51 (95% CI 1.15–1.95) mg dl^{-1} when all the other covariates remained constant. The proportion of European admixture was available for 83% of the CLEAR 2 participants included in the analysis. After adjusting for the proportion of European admixture, the CRP genotype rs3093062 was associated with increased plasma CRP levels in CLEAR 2 (mean estimate (95% CI) 0.19 (0.07–0.32), $P = 0.0028$) (Table 5).

DISCUSSION

This is the first study to evaluate the association of genetic variation in *CRP* locus with joint damage in RA and is focused on an important minority racial/ethnic group. We found that rs2808630 in the *CRP* 3′ flanking region was associated with radiographic damage in early RA (CLEAR 1) and identified statistically significant associations between a *CRP* promoter region SNP, rs3093062, and higher levels of plasma CRP in African Americans with established RA. Our results are concordant with a recent report from Plant *et al.*,[22] which found that *CRP* rs3093062 was associated with increased plasma CRP in RA patients from the UK. However, in contrast to Rhodes *et al.*,[13] we did not find an association between *CRP* SNPs rs1205 and plasma CRP levels in early RA, possibly due to differences in ethnicity (UK, New Zealand and Australia). Most importantly, we found that the *CRP* locus influences both radiographic damage and plasma CRP levels at different stages of the disease. A possible explanation for the finding of different *CRP* SNPs associated with phenotypes in early

Table 2. *CRP* genotypes associations with total radiographic score in African Americans from CLEAR 1

Variable	P-*value*	N	*Multivariate A*[a]			P-*value*	N	*Multivariate B*[b]			P-*value*
			IRR	*95% CI*				*IRR*	*95% CI*		
rs1417938	0.1962	288	0.71	0.35	1.45	0.3454	201	1.07	0.42	2.73	0.8882
rs2808630	0.0011	287	0.37	0.19	0.74	**0.0051**	200	0.43	0.19	0.97	0.0432
rs1205	0.9257	288	1.27	0.68	2.38	0.4499	201	1.5	0.72	3.12	0.2814
rs3093066	0.0546	288	1.99	1.00	3.96	0.0497	201	1.55	0.70	3.42	0.2808
rs3093059	0.0088	288	2.13	1.13	4.00	0.0191	201	1.61	0.75	3.45	0.2205
rs3093062	0.5815	288	1.09	0.50	2.40	0.8224	201	0.67	0.25	1.76	0.4157
rs3091244A	0.0017	286	2.40	1.26	4.59	0.0080	200	2.26	1.00	5.08	0.0486
rs3091244T	0.7506	286	1.07	0.57	2.02	0.8379	200	1.16	0.55	2.45	0.6951

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; IRR, incident rate ratio; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope. [a]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, disease duration, RF and ACPA status, plasma CRP level and DMARDs use. Statistically significant differences (as defined by Bonferroni corrected *P*-value < 0.0071) are in bold characters. [b]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, disease duration, RF and ACPA status, plasma CRP level, DMARDs use and European admixture.

Table 3. *CRP* genotypes associations with total radiographic score in African Americans from CLEAR 2

Variable	*Univariate* P-*value*	N	*Multivariate A*[a]			P-*value*	N	*Multivariate B*[b]			P-*value*
			IRR	*95% CI*				*IRR*	*95% CI*		
rs1417938	0.4673	134	1.43	0.84	2.42	0.1856	123	1.39	0.83	2.34	0.2151
rs2808630	0.6553	392	1.28	0.91	1.80	0.1599	327	1.18	0.81	1.71	0.3970
rs1205	0.6733	392	0.89	0.65	1.24	0.4989	327	0.80	0.58	1.11	0.1755
rs3093066	0.9973	392	1.00	0.73	1.38	0.9762	327	1.08	0.78	1.50	0.6459
rs3093059	0.8990	392	0.98	0.72	1.34	0.9105	327	1.08	0.78	1.49	0.6339
rs3093062	0.3777	392	0.81	0.56	1.16	0.2444	327	0.84	0.57	1.24	0.3754
rs3091244A	0.0772	132	0.88	0.52	1.47	0.6146	120	0.87	0.51	1.47	0.5914
rs3091244T	0.4601	132	0.94	0.60	1.47	0.7968	120	0.88	0.56	1.39	0.5886

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; IRR, incident rate ratio; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope. [a]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, disease duration, RF and ACPA status, plasma CRP level, glucocorticoids use, DMARDs use, and number of tender joints. Statistically significant (as defined by Bonferroni corrected *P*-value < 0.0071) differences are in bold characters. [b]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, disease duration, RF and ACPA status, plasma CRP level, glucocorticoids use, DMARDs use, number of tender joints and European admixture.

Table 4. *CRP* genotypes associations with log transformed plasma CRP in African American from CLEAR 1

Variable	*Univariate* P-*value*	N	*Multivariate A*[a]			P-*value*	N	*Multivariate B*[b]			P-*value*
			β	*95% CI*				*β*	*95% CI*		
rs1417938	0.2089	203	−0.14	−0.37	0.08	0.2202	147	−0.19	−0.49	0.11	0.2207
rs2808630	0.5439	202	−0.01	−0.21	0.18	0.912	146	−0.01	−0.26	0.24	0.9369
rs1205	0.0136	203	−0.24	−0.41	−0.06	0.0095	147	−0.22	−0.44	0	0.0552
rs3093066	0.8573	203	0.01	−0.15	0.17	0.8854	147	0.04	−0.16	0.23	0.7198
rs3093062	0.0005	203	0.22	0.05	0.40	0.0129	147	0.08	−0.10	0.27	0.3789
rs3093059	0.4140	203	0.05	−0.1	0.20	0.5418	147	0.21	−0.02	0.44	0.0710
rs3091244A	0.0933	201	0.09	−0.08	0.26	0.2983	145	0.12	−0.09	0.33	0.2626
rs3091244T	0.0102	201	0.16	−0.01	0.32	0.0594	145	0.13	−0.08	0.34	0.2146

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; RF, rheumatoid factor; SE, shared epitope. [a]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, RF and ACPA status, corticosteroid use, number of swollen joints, number of tender joints. Statistically significant differences (as defined by Bonferroni corrected *P*-value < 0.0071) are in bold characters. [b]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, RF and ACPA status, corticosteroid use, number of swollen joints, number of tender joints, European admixture.

and established disease is that there are significant differences in the clinical and demographic characteristics between the two groups analyzed, early vs established. Alternatively, it is possible that the pathobiology of early RA is different than that of established RA (that is, the activity of different transcription factors is affected by *CRP* SNPs in early versus established RA). In an attempt to test this theory, we analyzed the association of rs2808630 and total radiographic scores in 60 CLEAR 2 participants with disease duration of less than 2 years. We found that the CRP rs2908630 was not associated with less radiographic damage

Table 5. *CRP* genotypes associations with log transformed plasma CRP in African Americans from CLEAR 2

Variable	*Univariate* P-*value*	N	*Multivariate A*[a]			P-*value*	N	*Multivariate B*[b]			P-*value*
			β	*95% CI*				*β*	*95% CI*		
rs1417938	0.7516	139	−0.01	−0.2	0.19	0.9562	127	−0.05	−0.25	0.15	−0.48
rs2808630	0.0232	420	−0.13	−0.25	−0.02	0.0232	347	−0.15	−0.28	−0.02	−2.25
rs1205	0.6904	420	−0.08	−0.19	0.02	0.1305	347	−0.07	−0.20	0.05	−1.22
rs3093066	0.8283	420	0.01	−0.1	0.12	0.8478	347	0.01	−0.12	0.13	0.08
rs3093062	0.0050	420	0.18	0.06	0.29	**0.0021**	347	0.19	0.07	0.32	3.01
rs3093059	0.9645	420	0.03	−0.07	0.13	0.6041	347	0.04	−0.08	0.15	0.62
rs3091244A	0.1592	137	−0.12	−0.32	0.09	0.2567	124	−0.08	−0.30	0.13	−0.76
rs3091244T	0.0509	137	0.18	0.03	0.34	0.0189	124	0.14	−0.03	0.3	1.65

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; RF, rheumatoid factor; SE, shared epitope. [a]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, RF and ACPA status, corticosteroid use, number of swollen joints, number of tender joints. Statistically significant differences (as defined by Bonferroni corrected *P*-value < 0.0071) are in bold characters. [b]Additive model minor allele (0 vs 1 vs 2) after adjusting for age, gender, BMI, RF and ACPA status, corticosteroid use, number of swollen joints, number of tender joints, European admixture. Statistically significant differences (as defined by Bonferroni corrected *P*-value < 0.0071) are in bold characters.

(incident rate ratio = 0.61, *P* = NS), but the direction of the effect was similar to the results obtained in CLEAR 1. This result suggests that future studies of *CRP* genetic variation and radiographic severity are warranted in early RA.

A major strength of this study is that it analyzes the largest group of African Americans with RA available to date. Several clinical (age, sex, disease duration, medication use, disease activity), laboratory (autoantibody positivity, CRP level, erythrocyte sedimentation rate), genetic (*HLA DRB1* shared epitope) and environmental (smoking) factors previously reported to contribute to structural joint damage in RA.[23,24] This paper is the first to provide evidence of a *CRP* genetic contribution to structural joint damage in African Americans with RA, independent of age, gender, disease duration, DMARD use and autoantibody status (RF and ACPA status). The association of *CRP* SNP rs2808630 with lower radiographic damage has biological plausibility, because of previous literature supporting an association between this variant and lower CRP levels in a much larger sample of African Americans (~2100) from the Third National Health and Nutrition Examination Survey (NHANES III).[10]

Previous studies have shown that the radiographic damage in RA is heritable, with the h^2 estimate ranging from 45–58%.[25] Our study adds to the growing body of evidence in support of genetic associations with radiographic severity in multiple populations: *IL1* cluster,[26] *TNFa*,[27,28] *IL4R*,[29,30] *TRAF1-C5*, *TNFAIP3-OLIG3*,[31,32] 6q23 region, *IL10*, *IL6*, *CD40*,[31,33–35] *MMP3*,[36] *PTPN22*, *RANKL* and *IL17*.[37] There is mixed support in the literature for a correlation between *HLA-DRB1* SE alleles and radiographic damage.[28,37–40 27] We did not specifically include any of these non-HLA loci in our models, but we did include HLA-DRB1 SE status and did not find an association of SE status and radiographic severity. This is consistent with findings of Khanna *et al.*[27] in a study of Caucasians with early RA. In our study, the absence of association between SE alleles and radiographic severity may be because of a lower frequency of *HLA-DRB1* SE alleles in African Americans compared with European ancestry RA.[41]

The present study represents a candidate SNP approach aimed at defining the contribution of seven *CRP* polymorphisms to joint damage in RA. Future fine mapping of *CRP* may identify more genetic variants linked to radiographic severity and plasma CRP levels in African Americans with RA. Because our study included only African Americans, the association of *CRP* variants with radiographic severity is not generalizable to the entire population of patients with RA and further studies in other ethnicities are needed to address this issue.

In summary, among African Americans with early RA after taking in consideration clinical relevant factors, *CRP* rs2808630 is associated with lower radiographic damage, while *CRP* rs3093059 is associated with increased plasma CRP levels in African Americans with RA independent of other factors. These findings have important implications for assessment of disease activity and risk of erosive disease in African Americans with early RA.

MATERIALS AND METHODS

Study population

The CLEAR 1 Registry enrolled African Americans with RA between 2000 and 2005 and included only patients with less than 2 years disease duration. CLEAR 2 enrolled African Americans with RA between 2006 and 2011 and included RA of any disease duration.[20] Patients enrolled in CLEAR 1 were not enrolled in CLEAR 2, so there was no overlap in the patient populations. All participants met revised 1987 American Rheumatism Association (now the American College of Rheumatology) criteria,[42] and were self-declared African Americans of 19 years of age and older. After signing informed consent, the participants were recruited in Alabama (University of Alabama at Birmingham—Coordinating Center), Georgia (Emory University), North Carolina (University of North Carolina at Chapel Hill), Missouri (Washington University) and South Carolina (The Medical University of South Carolina). Peripheral blood was collected for measurement of RF, ACPA, plasma CRP and for isolation of genomic DNA. Questionnaires were administered to document current and previous drug treatments with DMARDs and corticosteroids. We included in this analysis sets of page#11hand/wrists (postero-anterior views) and feet (anteroposterior views) radiographs obtained at enrollment and scored using the modified Sharp/van der Heijde scoring system and assigned a mTSS.[20,43] Participants provided written informed consent and human subject protocols were approved by the Institutional Review Boards of the participating institutions.

The following socio-demographic variables were included as covariates in the analyses: age at enrollment, sex, disease duration, body mass index (BMI) and smoking status (ever vs never). The clinical variables included were: tender and swollen joint counts (assessed in 28 joints—shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and knees), usage of methotrexate and other DMARDs, biological agents and corticosteroids.

CRP levels ($mg\,l^{-1}$) were measured on plasma samples from the enrollment visits at the Clinical and Epidemiological Research Laboratory at Children's Hospital in Boston using a high-sensitivity immunoturbidimetric assay on a Hitachi 917 autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA), with the use of reagents and calibrators from Denka Seiken (Tokyo, Japan). High levels of CRP were defined as $> 3\,mg\,l^{-1}$.[44] RF and ACPA were assayed as previously reported.[45] We measured plasma rather than serum CRP levels because of the availability of plasma specimens for our study population and because the measurement of CRP in plasma and serum are comparable.[46]

Table 6. *CRP* variants studied in African Americans with rheumatoid arthritis

Marker	*Position (HG37.3)*	*HWE P-value*	*Location*	*MAF*	*Alleles*[a]
rs2808630	159 680 868	0.82	3' Flanking	0.16	T:C
rs1205	159 682 233	0.69	3' Flanking	0.20	C:T
rs3093066	159 683 099	1.00	3' UTR	0.20	G:T
rs1800947	159 683 438	1.00	Exon 2	0.01	C:G
rs1417938	159 684 186	0.90	Intron 1	0.13	T:A
rs3091244	159 684 665	0.75	Promoter	0.32 (T) 0.23 (A)	C:T:A
rs3093062	159 684 684	0.98	Promoter	0.19	G:A
rs3093061	159 684 982	0.33	Promoter	0.19	A:G
rs2794521	159 685 096	0.43	Promoter	0.14	A:G
rs3093059	159 685 136	0.73	Promoter	0.23	A:G
rs3093058	159 685 315	0.80	Promoter	0.19	T:A

Abbreviations: CRP, C-reactive protein; HG, human genome; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; UTR, untranslated region. [a]Alleles are reported in order from most frequent to least frequent. Depending on the DNA strand sequenced, allele designations may differ from other reports in the literature.

Genotyping methods

Eleven *CRP* SNPs (rs3093058, rs3093059, rs2794521, rs3093061, rs3093062, rs3091244, rs1417938, rs1800947, rs3093066, rs1205 and rs2808630) were selected for genotyping based on several criteria at the time of study inception: disease associations reported in the literature; MAF > 5% in published databases; or potential biological function[10,11,18,47,48] (see Table 6). For 347 CLEAR 1 participants and 143 CLEAR 2 participants, nine of the *CRP* SNPs were genotyped using Applied Biosystems (ABI) TaqMan Genotyping Assays, (Foster City, CA, USA) on an ABI 7900HT Sequence Detection System with a call rate > 99%. The tri-allelic SNP rs3091244 SNP and the biallelic SNP rs3093061 were genotyped by Pyrosequencing[49] using Biotage (Charlotte, NC, USA) reagents and protocol; the call rate for both was 99%. For 440 CLEAR 2 participants, genotype data of rs3093059, rs2794521, rs3093062, rs3093066, rs1205, rs2808630 were available as part a of a previous genotyping effort through the Immunochip (iChip) custom array by Illumina (San Diego, CA, USA); these markers had a call rate > 98.5%. One *CRP* polymorphism (rs1800947) had a MAF of 0.005 and thus was not included in the analysis. *HLA DRB1* genotyping was performed using the Atria Genetics (South San Francisco, CA, USA) AlleleSEQR DRB1 reagents and protocol as previously described.[41]

Statistical methods

Means (s.d.) were calculated for normally distributed continuous variables and proportions were calculated for categorical variables. Student *t*-tests and X^2-tests were used to evaluate differences between continuous and categorical variables, respectively. Linkage disequilibrium between biallelic SNP pairs was analyzed using Haploview[50] (Supplementary Figure 1). *CRP* polymorphisms rs3093061, rs3093058, and rs3093062 were in tight linkage disequilibrium (pairwise $r^2 > 0.8$), as were rs2808630 and rs2794521; only one *CRP* SNP from each of these two sets (rs3093062 and rs2808630) was included in the data analysis. As noted above, one *CRP* polymorphism (rs1800947) had a MAF of < 1% and was excluded from the data analysis. Genotype data for *CRP* SNPs rs3091244 and rs1417938 was available for analysis in only 140 (~34%) CLEAR 2 participants. There were no significant differences in the MAF of the CRP SNPs between CLEAR 1 and CLEAR 2 (data not shown). Deviation from Hardy–Weinberg equilibrium for each of the *CRP* polymorphisms was assessed using Haploview. All the *CRP* SNPs analyzed were in Hardy–Weinberg equilibrium.

We performed separate data analysis in the two study groups: CLEAR 1 and CLEAR 2 for several reasons, including different enrollment periods (2000–2005 for CLEAR 1; 2006–2011 for CLEAR 2), different available medications, different disease duration and differences in degree of radiographic damage. Complete data on genotypes, radiographic scores and CRP measurements were available for 294 (83%) of the 355 CLEAR 1 participants and 407 (57%) of the 712 CLEAR 2 participants. There were no statistically significant differences in clinical and biochemical characteristics between the persons for whom radiographs were available and the entire study population (data not shown).

The mTSS for these two groups of patients was overdispersed, with the majority having no damage (mTSS = 0) in 200 (68%) of CLEAR 1, and 248 (61%) of CLEAR 2 participants. Thus, negative binomial models were fitted to evaluate the association of *CRP* genotypes (explanatory variable) with the mTSS (dependent variable) in an additive genetic model framework. Given the positively skewed distribution of the plasma CRP levels, these values were log transformed for the purposes of the analysis. Linear regression was used to investigate the relationship between *CRP* genotypes and log transformed plasma CRP levels, assuming an additive genetic model of inheritance. Demographic and clinical variables with *P*-value < 0.25 in the univariate analyses were included in the multivariate analyses.[51] In addition, since gender, CRP levels and traditional and biological DMARD use have been shown to affect the progression of radiographic joint damage, these covariates were included in multivariable models with mTSS as the outcome variable. The final multivariable models were adjusted for the proportion of European admixture, which was available for 69% of the participants in CLEAR 1 and 83% individuals in CLEAR 2 as part a of a previous genotyping effort through the Immunochip (iChip) custom array by Illumina. Bonferroni correction was used to adjust for multiple comparisons for both the primary (mTSS) and secondary (log transformed CRP level) outcomes. After removing SNPs with low MAF and those in tight linkage disequilibrium from the analysis (see Materials and methods), seven independent SNPs remained. Thus, for the association analysis, we considered statistical significance to be achieved at the $\alpha = 0.05/7 = 0.0071$.

CONFLICT OF INTEREST

DM van der Heijde is Director of Imaging Rheumatology BV, The Netherlands. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The work reported in the manuscript was supported by the UAB Center for Clinical and Translational Studies (grant number 5UL1 RR025777-03 from the NIH National Center for Research Resources; NIH N01 AR-6-2278—Continuation of the Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis (CLEAR) Registry); NIH 2P60 AR048095-06 Multidisciplinary Clinical Research Center Project 3: Predictors of Rheumatoid Arthritis Severity in African Americans (SLB); and NIH K23 AR62100 (MID), NIH K01 AR060848 (RJR) and an American College of Rheumatology Research and Education Foundation Within Our Reach Rheumatoid Arthritis Translational Research Grant (SLB). The study sponsors were not involved in the study design, the collection, analysis and interpretation data, the writing of the report or in the decision to submit the paper for publication. We acknowledge the contribution of the following CLEAR Investigators: Beth L Jonas, MD, University of North Carolina, Chapel Hill, NC, USA; Edwin A Smith, MD, Medical University of South Carolina, Charleston, SC, USA; Richard D Brasington, Jr, MD, Washington University, St. Louis, MO, USA for providing access to data for this study. We thank Peter K Gregersen, MD at The Feinstein Institute for Medical Research, Manhasset, NY, USA, for providing access to previous immunochip genotyping data for this study.

REFERENCES

1 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011; **365**: 2205–2219.
2 Walters MT, Stevenson FK, Goswami R, Smith JL, Cawley MI. Comparison of serum and synovial fluid concentrations of beta 2-microglobulin and C reactive protein in relation to clinical disease activity and synovial inflammation in rheumatoid arthritis. *Ann Rheum Dis* 1989; **48**: 905–911.
3 Zeller JM, Kubak BM, Gewurz H. Binding sites for C-reactive protein on human monocytes are distinct from IgG Fc receptors. *Immunology* 1989; **67**: 51–55.
4 Potempa LA, Zeller JM, Fiedel BA, Kinoshita CM, Gewurz H. Stimulation of human neutrophils, monocytes, and platelets by modified C-reactive protein (CRP) expressing a neoantigenic specificity. *Inflammation* 1988; **12**: 391–405.
5 James K, Baum LL, Vetter ML, Gewurz H. Interactions of C-reactive protein with lymphoid cells. *Ann N Y Acad Sci* 1982; **389**: 274–285.
6 Mallya RK, Vergani D, Tee DE, Bevis L, de Beer FC, Berry H *et al*. Correlation in rheumatoid arthritis of concentrations of plasma C3d, serum rheumatoid factor, immune complexes and C-reactive protein with each other and with clinical features of disease activity. *Clin Exp Immunol* 1982; **48**: 747–753.
7 Inoue E, Yamanaka H, Hara M, Tomatsu T, Kamatani N. Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28- C-reactive protein threshold values. *Ann Rheum Dis* 2007; **66**: 407–409.

8 Ranganath VK, Khanna D, Paulus HE. ACR remission criteria and response criteria. *Clin Exp Rheumatol* 2006; **24**(6 Suppl 43): S-14–S-21.
9 Wolfe F, Pincus T. The level of inflammation in rheumatoid arthritis is determined early and remains stable over the longterm course of the illness. *J Rheumatol* 2001; **28**: 1817–1824.
10 Crawford DC, Sanders CL, Qin X, Smith JD, Shephard C, Wong M *et al.* Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. *Circulation* 2006; **114**: 2458–2465.
11 Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A *et al.* Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. *J Mol Med* 2005; **83**: 440–447.
12 Lange LA, Carlson CS, Hindorff LA, Lange EM, Walston J, Durda JP *et al.* Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. *JAMA* 2006; **296**: 2703–2711.
13 Rhodes B, Merriman ME, Harrison A, Nissen MJ, Smith M, Stamp L *et al.* A genetic association study of serum acute-phase C-reactive protein levels in rheumatoid arthritis: implications for clinical interpretation. *PLoS Med* 2010; **7**: e1000341.
14 Hung AM, Crawford DC, Griffin MR, Brown-Gentry K, Lipkowitz MS, Siew ED *et al.* CRP polymorphisms and progression of chronic kidney disease in African Americans. *Clin J Am Soc Nephrol* 2010; **5**: 24–33.
15 Kuhlenbaeumer G, Huge A, Berger K, Kessler C, Voelzke H, Funke H *et al.* Genetic variants in the C-reactive protein gene are associated with microangiopathic ischemic stroke. *Cerebrovasc Dis* 2010; **30**: 476–482.
16 Kok EH, Alanne-Kinnunen M, Isotalo K, Luoto T, Haikonen S, Goebeler S *et al.* CRP gene variation affects early development of Alzheimer's disease-related plaques. *J Neuroinflammation* 2011; **8**: 96.
17 Yang SH, Huang CJ, Chang SC, Lin JK. Association of C-reactive protein gene polymorphisms and colorectal cancer. *Ann Surg Oncol* 2011; **18**: 1907–1915.
18 Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M *et al.* Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet* 2005; **77**: 64–77.
19 Machold KP, Stamm TA, Nell VP, Pflugbeil S, Aletaha D, Steiner G *et al.* Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. *Rheumatology (Oxford)* 2007; **46**: 342–349.
20 Bridges SL Jr., Causey ZL, Burgos PI, Huynh BQ, Hughes LB, Danila MI *et al.* Radiographic severity of rheumatoid arthritis in African Americans: results from a multicenter observational study. *Arthritis Care Res (Hoboken)* 2010; **62**: 624–631.
21 Tamhane A, Redden DT, McGwin G Jr., Brown EE, Westfall AO, RJ Reynolds 4th *et al.* Comparison of the disease activity score using erythrocyte sedimentation rate and C-reactive protein in African Americans with rheumatoid arthritis. *J Rheumatol* 2013; **40**: 1812–1822.
22 Plant D, Ibrahim I, Lunt M, Eyre S, Flynn E, Hyrich KL *et al.* Correlation of C-reactive protein haplotypes with serum C-reactive protein level and response to anti-tumor necrosis factor therapy in UK rheumatoid arthritis patients: results from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate cohort. *Arthritis Res Ther* 2012; **14**: R214.
23 Syversen SW, Gaarder PI, Goll GL, Odegard S, Haavardsholm EA, Mowinckel P *et al.* High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 2008; **67**: 212–217.
24 Vastesaeger N, Xu S, Aletaha D St, Clair EW, Smolen JS. A pilot risk model for the prediction of rapid radiographic progression in rheumatoid arthritis. *Rheumatology (Oxford)* 2009; **48**: 1114–1121.
25 Knevel R, Grondal G, Huizinga TW, Visser AW, Jonsson H, Vikingsson A *et al.* Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. *Ann Rheum Dis* 2012; **71**: 707–709.
26 Cox A, Camp NJ, Cannings C, di Giovine FS, Dale M, Worthington J *et al.* Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis. *Hum Mol Genet* 1999; **8**: 1707–1713.
27 Khanna D, Wu H, Park G, Gersuk V, Gold RH, Nepom GT *et al.* Association of tumor necrosis factor alpha polymorphism, but not the shared epitope, with increased radiographic progression in a seropositive rheumatoid arthritis inception cohort. *Arthritis Rheum* 2006; **54**: 1105–1116.
28 Reneses S, Gonzalez-Escribano MF, Fernandez-Suarez A, Pestana L, Davila B, Wichmann I *et al.* The value of HLA-DRB1 shared epitope, -308 tumor necrosis factor-alpha gene promoter polymorphism, rheumatoid factor, anti-citrullinated peptide antibodies, and early erosions for predicting radiological outcome in recent-onset rheumatoid arthritis. *J Rheumatol* 2009; **36**: 1143–1149.
29 Prots I, Skapenko A, Wendler J, Mattyasovszky S, Yone CL, Spriewald B *et al.* Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 1491–1500.
30 Burgos PI, Causey ZL, Tamhane A, Kelley JM, Brown EE, Hughes LB *et al.* Association of IL4R single-nucleotide polymorphisms with rheumatoid nodules in African Americans with rheumatoid arthritis. *Arthritis Res Ther* 2010; **12**: R75.
31 Scherer HU, van der Linden MP, Kurreeman FA, Stoeken-Rijsbergen G, Cessie S, Huizinga TW *et al.* Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis. *Ann Rheum Dis* 2010; **69**: 567–570.
32 Knevel R, de Rooy DP, Gregersen PK, Lindqvist E, Wilson AG, Grondal G *et al.* Studying associations between variants in TRAF1-C5 and TNFAIP3-OLIG3 and the progression of joint destruction in rheumatoid arthritis in multiple cohorts. *Ann Rheum Dis* 2012; **71**: 1753–1755.
33 Lard LR, van Gaalen FA, Schonkeren JJ, Pieterman EJ, Stoeken G, Vos K *et al.* Association of the -2849 interleukin-10 promoter polymorphism with autoantibody production and joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2003; **48**: 1841–1848.
34 van der Linden MP, Feitsma AL, le Cessie S, Kern M, Olsson LM, Raychaudhuri S *et al.* Association of a single-nucleotide polymorphism in CD40 with the rate of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2009; **60**: 2242–2247.
35 Marinou I, Healy J, Mewar D, Moore DJ, Dickson MC, Binks MH *et al.* Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. *Arthritis Rheum* 2007; **56**: 2549–2556.
36 Nemec P, Pavkova-Goldbergova M, Gatterova J, Vasku A, Soucek M. Association of the 5 A/6 A promoter polymorphism of the MMP-3 gene with the radiographic progression of rheumatoid arthritis. *Ann N Y Acad Sci* 2007; **1110**: 166–176.
37 Furuya T, Hakoda M, Ichikawa N, Higami K, Nanke Y, Yago T *et al.* Associations between HLA-DRB1, RANK, RANKL, OPG, and IL-17 genotypes and disease severity phenotypes in Japanese patients with early rheumatoid arthritis. *Clin Rheumatol* 2007; **26**: 2137–2141.
38 van Zeben D, Hazes JM, Zwinderman AH, Cats A, Schreuder GM, D'Amaro J *et al.* Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis. Results of a followup study. *Arthritis Rheum* 1991; **34**: 822–830.
39 Reveille JD, Alarcon GS, Fowler SE, Pillemer SR, Neuner R, Clegg DO *et al.* HLA-DRB1 genes and disease severity in rheumatoid arthritis. The MIRA Trial Group. Minocycline in Rheumatoid Arthritis. *Arthritis Rheum* 1996; **39**: 1802–1807.
40 Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H *et al.* HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. *Arthritis Rheum* 1997; **40**: 341–351.
41 Hughes LB, Morrison D, Kelley JM, Padilla MA, Vaughan LK, Westfall AO *et al.* The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis Rheum* 2008; **58**: 349–358.
42 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315–324.
43 van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000; **27**: 261–263.
44 Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M *et al.* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107**: 499–511.
45 Mikuls TR, Holers VM, Parrish L, Kuhn KA, Conn DL, Gilkeson G *et al.* Anti-cyclic citrullinated peptide antibody and rheumatoid factor isotypes in African Americans with early rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 3057–3059.
46 Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol* 2003; **10**: 652–657.
47 Rhodes B, Morris DL, Subrahmanyan L, Aubin C, de Leon CF, Kelly JF *et al.* Fine-mapping the genetic basis of CRP regulation in African Americans: a Bayesian approach. *Hum Genet* 2008; **123**: 633–642.
48 Edberg JC, Wu J, Langefeld CD, Brown EE, Marion MC, McGwin G Jr. *et al.* Genetic variation in the CRP promoter: association with systemic lupus erythematosus. *Hum Mol Genet* 2008; **17**: 1147–1155.
49 Ronaghi M, Uhlen M, Nyren P. A sequencing method based on real-time pyrophosphate. *Science* 1998; **281**(5375): 363–365.
50 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
51 Hosmer DW, Lemeshow S. *Applied Logistic Regression*. Wiley: New York, NY, USA, 2000.

Supplementary Information accompanies this paper on Genes and Immunity website (http://www.nature.com/gene)