Heterologous Expression and Functional Characterization of the Exogenously Acquired Aminoglycoside Resistance Methyltransferases RmtD, RmtD2, and RmtG

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The Role of Genetic Variants in CRP in Radiographic Severity in African Americans with Early and Established Rheumatoid Arthritis

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Abstract

This study investigates the association of CRP 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 in 294 individuals from CLEAR 1 [median (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 (IRR) 0.37 (95% 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 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.

Keywords

C reactive protein; genetics; rheumatoid arthritis; joint damage; radiography

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CONFLICT OF INTEREST: None
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. Although the biological processes underlying the pathophysiology of RA are not completely understood, systemic inflammation, as reflected by serum CRP levels, is thought to represent a key component. CRP is found in the synovial fluid of RA patients and can bind to lymphocytes, monocytes and other inflammatory cells. Serum CRP level is commonly used to assess disease activity in RA patients and is part of the Disease Activity Score in 28 joints (DAS28-CRP). Serum CRP levels are also included in the American College of Rheumatology (ACR) treatment response criteria. 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.

Single nucleotide polymorphisms (SNPs) in CRP have been shown to be associated with serum CRP levels and their biologic role has been evaluated in different disease states including RA, cardiovascular disease, Alzheimer’s disease, colorectal cancer, and chronic kidney disease. 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.

There is great variability in the minor allele frequency (MAF) of CRP SNPs among different ethnic groups. CRP SNP rs3093058 is part of a haplotype associated with incident stroke in African-Americans, but this SNP is monomorphic (MAF=0) in European-Americans and thus does not contribute to the risk of stroke in that ethnic group. 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. 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. 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 longstanding disease to evaluate whether systemic inflammation and RA-induced joint damage have common genetic determinants.
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. 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 longer disease duration, a lower median tender joint count, were more likely to be autoantibody positive (RF and ACPA). In addition, there were differences in use of DMARDs and biologic agents. Because enrollment in CLEAR 1 occurred between 2000 and 2005, a relatively low percentage (~4%) of participants used biologic agents (etanercept, infliximab and anakinra). Enrollment in CLEAR 2 occurred between 2006 and 2011, so a higher percentage (~36%) of participants had been on biologic 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. The Pearson correlation between the CRP SNPs and DAS28-3(CRP) ranged from −0.09 and 0.09. Since global health scores by visual analog scale were available in CLEAR 2, we were able to calculate DAS28(CRP) with 4 variables. The Pearson correlation between CRP SNPs and DAS28(CRP) ranged from −0.12 and 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 (IQR 25-75) mTSS at enrollment in CLEAR 1 was 0 (0–2), while in CLEAR 2, the median (IQR 25-75) mTSS was 6 (0–31), as previously reported. 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 3 and 4. 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 biologic DMARD [IRR 0.37 (95% CI 0.19-0.74), p value=0.0051] (Table 3). 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 (SD) 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 [IRR 0.43 (95% CI 0.19-0.97), p-value=0.0432] (Table 3). None of the CRP SNPs was associated with radiographic severity in CLEAR 2 after adjusting for relevant covariates (Table 4).

**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 5 and 6. 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 6). Thus, for each rs3093062 A minor allele, the plasma CRP increased by 1.51 (95% CI 1.15-1.95) mg/dL when all the other covariates remained constant. The proportion of European admixture was available for 83% of the CLEAR2 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 6).

**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 Australians). 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 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 (i.e. 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 (IRR =0.61, p=NS), but the direction of the effect was similar to the results obtained in CLEAR1. 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, ESR), genetic (HLA DRB1 shared epitope) and environmental (smoking) factors previously reported to contribute to structural joint damage in RA. 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 (~2,100) from the Third National Health and Nutrition Examination Survey (NHANES III).

Previous studies have shown that the radiographic damage in RA is heritable, with the $h^2$ estimate ranging from 45 to 58%. Our study adds to the growing body of evidence in support of genetic associations with radiographic severity in multiple populations: IL1 cluster, TNFa, IL4R, TRAF1-C5, TNFAIP3-OLIG3, 6q23 region, IL10, IL6, CD40, CD22, PTPN22, RANKL, IL17. There is mixed support in the literature for a correlation between HLA-DRB1 SE alleles and radiographic damage. 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. in a study of Caucasians with early RA. In our study, the absence of association between SE alleles and radiographic severity may be due to a lower frequency of HLA-DRB1 SE alleles in African Americans compared to European ancestry RA.

The present study represents a candidate SNP approach aimed at defining the contribution of 7 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. 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, 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 rheumatoid factor (RF), anti-
cyclic citrullinated peptide antibodies (ACPA), plasma CRP and for isolation of genomic
DNA. Questionnaires were administered to document current and previous drug treatments
with disease-modifying anti-rheumatic drugs (DMARDs) and corticosteroids. We included
in this analysis sets of page#1 hand/wrists (postero-anterior views) and feet (antero-
posterior views) radiographs obtained at enrollment and scored using the modified
Sharp/van der Heijde scoring system and assigned a modified total Sharp score
(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, biologic agents, and
corticosteroids.

CRP levels (mg/L) 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), with the use of reagents and calibrators from Denka Seiken
(Tokyo, Japan). High levels of CRP were defined as > 3 mg/L\(^{44}\). 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\(^{46}\).

Genotyping methods

Eleven CRP SNPs (rs3093058, rs3093059, rs2794521, rs3093061, rs3093062, rs3091244,
rs1417938, rs1800947, rs3093066, rs1205, rs2808630) were selected for genotyping based
on several criteria at the time of study inception: disease associations reported in the
literature; minor allele frequency > 5% in published databases; or potential biological
function\(^{10, 11, 18, 47, 48}\) (see Table 2). For 347 CLEAR 1 participants and 143 CLEAR 2
participants, 9 of the CRP SNPs were genotyped using Applied Biosystems (ABI) TaqMan\(^{®}\)
Genotyping Assays, (Foster City, CA) on an ABI 7900HT Sequence Detection System with
a call rate of greater than 99%. The tri-allelic SNP rs3091244 SNP and the bi-allelic SNP
rs3093061 were genotyped by Pyrosequencing\(^{49}\) using Biotage (Charlotte, NC) 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); these markers had a call rate greater than 98.5%. One CRP polymorphism
(rs1800947) had a MAF of 0.005 and thus was not included in the analysis. HLA DRBI
genotyping was performed using the Atria Genetics (South San Francisco, CA) AlleleSEQR DRB1 reagents and protocol as previously described ⁴¹.

**Statistical methods**

Means (SD) were calculated for normally distributed continuous variables and proportions were calculated for categorical variables. Student t tests and chi square tests were used to evaluate differences between continuous and categorical variables, respectively. Linkage disequilibrium (LD) between biallelic SNP pairs was analyzed using Haploview ⁵⁰ (Supplemental Figure 1). CRP polymorphisms rs3093061, rs3093058, and rs3093062 were in tight LD (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 HWE for each of the CRP polymorphisms was assessed using Haploview. All the CRP SNPs analyzed were in HWE.

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 modified total radiographic score (mTSS) for these two groups of patients was over-dispersed, 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 biologic 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 (San Diego, CA). 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 Methods), 7 independent SNPs remained. Thus, for the association analysis, we considered statistical significance to be achieved at the alpha = 0.05/7 = 0.0071.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**ACKNOWLEDGMENTS**

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The authors would like to acknowledge the contribution of the following CLEAR Investigators: Beth L. Jonas, MD, University of North Carolina, Chapel Hill, NC; Edwin A. Smith, MD, Medical University of South Carolina, Charleston, SC; 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, for providing access to previous immunochip genotyping data for this study.

**REFERENCES**


Table 1

Characteristics of African American participants with RA from CLEAR registry.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CLEAR 1 (N=294)</th>
<th>CLEAR 2 (N=407)</th>
</tr>
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<tr>
<td>Age, years, mean (SD)</td>
<td>50.7 (13.4)</td>
<td>56.4 (11.8)</td>
</tr>
<tr>
<td>Age at diagnosis, years, mean (SD)</td>
<td>49.6 (13.4)</td>
<td>44.9 (12.7)</td>
</tr>
<tr>
<td>BMI, kg/m², mean (SD)¹</td>
<td>31.3 (7.6)</td>
<td>31.9 (7.7)</td>
</tr>
<tr>
<td>Disease duration, years, median (IQR 25–75)</td>
<td>1 (0–1.6)</td>
<td>8.9 (3.5–17.7)</td>
</tr>
<tr>
<td>Swollen joint count, median (IQR 25–75)</td>
<td>4 (1–9)</td>
<td>5 (1–13)</td>
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<tr>
<td>Tender joint count, median (IQR 25–75)</td>
<td>7 (2–18)</td>
<td>6 (2–14)</td>
</tr>
<tr>
<td>Gender, female, N(%)</td>
<td>242 (82.3)</td>
<td>349 (85.8)</td>
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<tr>
<td>Smoking, ever, N(%)²</td>
<td>156 (53.2)</td>
<td>210 (51.6)</td>
</tr>
<tr>
<td>RF status, positive, N(%)³</td>
<td>204 (70.3)</td>
<td>328 (80.8)</td>
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<tr>
<td>ACPA status, positive, N (%)⁴</td>
<td>183 (63.1)</td>
<td>292 (71.9)</td>
</tr>
<tr>
<td>Shared epitope status, present, N (%)⁵</td>
<td>122 (41.5)</td>
<td>154 (37.9)</td>
</tr>
<tr>
<td>Corticosteroids, ever, N(%)⁶</td>
<td>154 (79.8)</td>
<td>379 (93.8)</td>
</tr>
<tr>
<td>Synthetic DMARD use, ever, N (%)⁷</td>
<td>247 (84)</td>
<td>388 (98.5)</td>
</tr>
<tr>
<td>Biologic DMARD use, ever, N (%)⁸</td>
<td>12 (3.9)</td>
<td>145 (35.9)</td>
</tr>
<tr>
<td>mTSS, median (IQR 25–75)</td>
<td>0 (0–2)</td>
<td>6 (0–31)</td>
</tr>
<tr>
<td>Percent European admixture, mean (SD)⁹</td>
<td>17 (10.6)</td>
<td>15.4 (9.3)</td>
</tr>
<tr>
<td>CRP, mg/dl, median (IQR 25–75)¹⁰</td>
<td>5.1 (1.8–9.4)</td>
<td>4.2 (1.5–8.9)</td>
</tr>
</tbody>
</table>

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; SD, standard deviation; SE, shared epitope;

¹ Missing for 17 persons;
² Missing for 1 person;
³ Missing for 5 persons;
⁴ Missing for 5 persons;
⁵ Missing for 1 persons;
⁶ Missing for 104 persons;
⁷ Missing for 2 persons;
⁸ Missing for 3 persons;
⁹ Missing for 159 persons;
¹⁰ Missing for 5 persons.

* Biologic DMARDs: etanercept, infliximab, adalimumab and anakinra.
**Table 2**

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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Position (HG37.3)</th>
<th>HWE p-value</th>
<th>Location</th>
<th>MAF</th>
<th>Alleles*</th>
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<td>rs2808630</td>
<td>159680868</td>
<td>0.82</td>
<td>3' Flanking</td>
<td>0.16</td>
<td>T:C</td>
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<td>rs1205</td>
<td>159682233</td>
<td>0.69</td>
<td>3' Flanking</td>
<td>0.20</td>
<td>C:T</td>
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<td>rs3093066</td>
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<td>1.00</td>
<td>3' UTR</td>
<td>0.20</td>
<td>G:T</td>
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<tr>
<td>rs1800947</td>
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<td>Exon 2</td>
<td>0.01</td>
<td>C:G</td>
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<tr>
<td>rs1417938</td>
<td>159684186</td>
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<td>Intron 1</td>
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<td>T:A</td>
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<td>0.75</td>
<td>Promoter</td>
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<td>C:T:A</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.23 (A)</td>
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<tr>
<td>rs3093062</td>
<td>159684684</td>
<td>0.98</td>
<td>Promoter</td>
<td>0.19</td>
<td>G:A</td>
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<td>Promoter</td>
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<tr>
<td>rs3093059</td>
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<td>Promoter</td>
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<tr>
<td>rs3093058</td>
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<td>0.80</td>
<td>Promoter</td>
<td>0.19</td>
<td>T:A</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; HG, human genome; MAF, minor allele frequency; UTR, untranslated region.

* 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.
### Table 3

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
<th>N</th>
<th>Multivariate A$</th>
<th>p value</th>
<th>N</th>
<th>Multivariate B$$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IRR</td>
<td>95 % CI</td>
<td></td>
<td>IRR</td>
<td>95 % CI</td>
</tr>
<tr>
<td>rs1417938</td>
<td>0.1962</td>
<td>288</td>
<td>0.71</td>
<td>0.35</td>
<td>1.45</td>
<td>0.3454</td>
<td>0.8882</td>
</tr>
<tr>
<td>rs2808630</td>
<td>0.0011</td>
<td>287</td>
<td>0.37</td>
<td>0.19</td>
<td>0.74</td>
<td>0.0051</td>
<td>0.0432</td>
</tr>
<tr>
<td>rs1205</td>
<td>0.9257</td>
<td>288</td>
<td>1.27</td>
<td>0.68</td>
<td>2.38</td>
<td>0.4499</td>
<td>0.2814</td>
</tr>
<tr>
<td>rs3093066</td>
<td>0.0546</td>
<td>288</td>
<td>1.99</td>
<td>1</td>
<td>3.96</td>
<td>0.0497</td>
<td>0.2808</td>
</tr>
<tr>
<td>rs3093059</td>
<td>0.0088</td>
<td>288</td>
<td>2.13</td>
<td>1.13</td>
<td>4</td>
<td>0.0191</td>
<td>0.2205</td>
</tr>
<tr>
<td>rs3093062</td>
<td>0.5815</td>
<td>288</td>
<td>1.09</td>
<td>0.5</td>
<td>2.4</td>
<td>0.8224</td>
<td>0.4157</td>
</tr>
<tr>
<td>rs3091244 A</td>
<td>0.0017</td>
<td>286</td>
<td>2.4</td>
<td>1.26</td>
<td>4.59</td>
<td>0.008</td>
<td>0.0486</td>
</tr>
<tr>
<td>rs3091244 T</td>
<td>0.7506</td>
<td>286</td>
<td>1.07</td>
<td>0.57</td>
<td>2.02</td>
<td>0.8379</td>
<td>0.6951</td>
</tr>
</tbody>
</table>

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 4

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate p value</th>
<th>N</th>
<th>IRR</th>
<th>95% CI</th>
<th>Multivariate A $^*$ p value</th>
<th>N</th>
<th>IRR</th>
<th>95% CI</th>
<th>Multivariate B $$$ p value</th>
<th>N</th>
<th>IRR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1417938</td>
<td>0.4673</td>
<td>134</td>
<td>1.43</td>
<td>0.84</td>
<td>2.42</td>
<td>0.1856</td>
<td>123</td>
<td>1.39</td>
<td>0.83</td>
<td>2.34</td>
<td>0.2151</td>
<td></td>
</tr>
<tr>
<td>rs2808630</td>
<td>0.6553</td>
<td>392</td>
<td>1.28</td>
<td>0.91</td>
<td>1.8</td>
<td>0.1599</td>
<td>327</td>
<td>1.18</td>
<td>0.81</td>
<td>1.71</td>
<td>0.397</td>
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</tr>
<tr>
<td>rs1205</td>
<td>0.6733</td>
<td>392</td>
<td>0.89</td>
<td>0.65</td>
<td>1.24</td>
<td>0.4989</td>
<td>327</td>
<td>0.8</td>
<td>0.58</td>
<td>1.11</td>
<td>0.1755</td>
<td></td>
</tr>
<tr>
<td>rs3093066</td>
<td>0.9973</td>
<td>392</td>
<td>1</td>
<td>0.73</td>
<td>1.38</td>
<td>0.9762</td>
<td>327</td>
<td>1.08</td>
<td>0.78</td>
<td>1.5</td>
<td>0.6459</td>
<td></td>
</tr>
<tr>
<td>rs3093059</td>
<td>0.899</td>
<td>392</td>
<td>0.98</td>
<td>0.72</td>
<td>1.34</td>
<td>0.9105</td>
<td>327</td>
<td>1.08</td>
<td>0.78</td>
<td>1.49</td>
<td>0.6339</td>
<td></td>
</tr>
<tr>
<td>rs3093062</td>
<td>0.3777</td>
<td>392</td>
<td>0.81</td>
<td>0.56</td>
<td>1.16</td>
<td>0.2444</td>
<td>327</td>
<td>0.84</td>
<td>0.57</td>
<td>1.24</td>
<td>0.3754</td>
<td></td>
</tr>
<tr>
<td>rs3091244A</td>
<td>0.0772</td>
<td>132</td>
<td>0.88</td>
<td>0.52</td>
<td>1.47</td>
<td>0.6146</td>
<td>120</td>
<td>0.87</td>
<td>0.51</td>
<td>1.47</td>
<td>0.5914</td>
<td></td>
</tr>
<tr>
<td>rs3091244T</td>
<td>0.4601</td>
<td>132</td>
<td>0.94</td>
<td>0.6</td>
<td>1.47</td>
<td>0.7968</td>
<td>120</td>
<td>0.88</td>
<td>0.56</td>
<td>1.39</td>
<td>0.5886</td>
<td></td>
</tr>
</tbody>
</table>

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.

$^*$ 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.

$$^$$ 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>
<thead>
<tr>
<th>Variable</th>
<th>Univariate p value</th>
<th>N</th>
<th>β</th>
<th>95 % CI</th>
<th>Multivariate A p value</th>
<th>N</th>
<th>β</th>
<th>95 % CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1417938</td>
<td>0.2089</td>
<td>203</td>
<td>-0.14</td>
<td>-0.37</td>
<td>0.08</td>
<td>2202</td>
<td>-0.19</td>
<td>-0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>rs2808630</td>
<td>0.5439</td>
<td>202</td>
<td>-0.01</td>
<td>-0.21</td>
<td>0.18</td>
<td>912</td>
<td>-0.01</td>
<td>-0.26</td>
<td>0.24</td>
</tr>
<tr>
<td>rs1205</td>
<td>0.0136</td>
<td>203</td>
<td>-0.24</td>
<td>-0.41</td>
<td>-0.06</td>
<td>0.0095</td>
<td>-0.22</td>
<td>-0.44</td>
<td>0</td>
</tr>
<tr>
<td>rs3093066</td>
<td>0.8573</td>
<td>203</td>
<td>0.01</td>
<td>-0.15</td>
<td>0.17</td>
<td>8854</td>
<td>0.04</td>
<td>-0.16</td>
<td>0.23</td>
</tr>
<tr>
<td>rs3093062</td>
<td>0.0005</td>
<td>203</td>
<td>0.22</td>
<td>0.05</td>
<td>0.4</td>
<td>0.0129</td>
<td>0.08</td>
<td>-0.1</td>
<td>0.27</td>
</tr>
<tr>
<td>rs3093059</td>
<td>0.414</td>
<td>203</td>
<td>0.05</td>
<td>-0.1</td>
<td>0.2</td>
<td>5418</td>
<td>0.21</td>
<td>-0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>rs3091244A</td>
<td>0.0933</td>
<td>201</td>
<td>0.09</td>
<td>-0.08</td>
<td>0.26</td>
<td>0.2983</td>
<td>0.12</td>
<td>-0.09</td>
<td>0.33</td>
</tr>
<tr>
<td>rs3091244T</td>
<td>0.0102</td>
<td>201</td>
<td>0.16</td>
<td>-0.01</td>
<td>0.32</td>
<td>0.0594</td>
<td>0.13</td>
<td>-0.08</td>
<td>0.34</td>
</tr>
</tbody>
</table>

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.
Table 6

CRP genotypes$\textsuperscript{a} associations with log transformed plasma CRP in African Americans from CLEAR 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>N</th>
<th>Multivariate A$</th>
<th>p value</th>
<th>N</th>
<th>Multivariate B$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td>β</td>
<td>95% CI</td>
<td></td>
<td>β</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>rs1417938</td>
<td>0.7516</td>
<td>0.01</td>
<td>-0.2</td>
<td>0.19</td>
<td>0.9562</td>
<td>0.05</td>
<td>-0.25</td>
</tr>
<tr>
<td>rs2808630</td>
<td>0.0232</td>
<td>-0.13</td>
<td>-0.25</td>
<td>-0.02</td>
<td>0.0232</td>
<td>0.15</td>
<td>-0.28</td>
</tr>
<tr>
<td>rs1205</td>
<td>0.6904</td>
<td>-0.08</td>
<td>-0.19</td>
<td>0.02</td>
<td>0.1305</td>
<td>-0.07</td>
<td>-0.2</td>
</tr>
<tr>
<td>rs3093066</td>
<td>0.8283</td>
<td>0.01</td>
<td>-0.1</td>
<td>0.12</td>
<td>0.8478</td>
<td>0.01</td>
<td>-0.12</td>
</tr>
<tr>
<td>rs3093062</td>
<td>0.005</td>
<td>0.18</td>
<td>0.06</td>
<td>0.29</td>
<td>0.0021</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>rs3093059</td>
<td>0.9645</td>
<td>0.03</td>
<td>-0.07</td>
<td>0.13</td>
<td>0.6041</td>
<td>0.04</td>
<td>-0.08</td>
</tr>
<tr>
<td>rs3091244A</td>
<td>0.1592</td>
<td>-0.12</td>
<td>-0.32</td>
<td>0.09</td>
<td>0.2567</td>
<td>-0.08</td>
<td>-0.3</td>
</tr>
<tr>
<td>rs3091244T</td>
<td>0.0509</td>
<td>0.18</td>
<td>0.03</td>
<td>0.34</td>
<td>0.0189</td>
<td>0.14</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

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

$\textsuperscript{a}$ 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.

$\textsuperscript{a}$ 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, European admixture. Statistically significant differences (as defined by Bonferroni corrected p value <0.0071) are in bold characters.