

# Genetic Variation in C-Reactive Protein (CRP) Gene May Be Associated with Risk of Systemic Lupus Erythematosus and CRP Concentrations

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**ABSTRACT. Objective.** The gene coding for C-reactive protein (CRP) is located on chromosome 1q23.2, which falls within a linkage region thought to harbor a systemic lupus erythematosus (SLE) susceptibility gene. Recently, 2 single-nucleotide polymorphisms (SNP) in the *CRP* gene (+838, +2043) have been shown to be associated with CRP concentrations and/or SLE risk in a British family-based cohort. Our study was done to confirm the reported association in an independent population-based case-control cohort, and also to investigate the influence of 3 additional *CRP* tagSNP (−861, −390, +90) on SLE risk and serum CRP concentrations.

**Methods.** DNA from 337 Caucasian women who met the American College of Rheumatology criteria for definite (n = 324) or probable (n = 13) SLE and 448 Caucasian healthy female controls was genotyped for 5 *CRP* tagSNP (−861, −390, +90, +838, +2043). Genotyping was performed using restriction fragment length polymorphism-polymerase chain reaction, pyrosequencing, or TaqMan assays. Serum CRP levels were measured using ELISA. Association studies were performed using the chi-squared distribution, Z-test, Fisher's exact test, and analysis of variance. Haplotype analysis was performed using EH software and the haplo.stats package in R 2.1.2.

**Results.** While none of the SNP were found to be associated with SLE risk individually, there was an association with the 5 SNP haplotypes (p < 0.001). Three SNP (−861, −390, +90) were found to significantly influence serum CRP level in SLE cases, both independently and as haplotypes.

**Conclusion.** Our data suggest that unique haplotype combinations in the *CRP* gene may modify the risk of developing SLE and influence circulating CRP levels. (First Release Sept 15 2008; J Rheumatol 2008;35:2171–8; doi:10.3899/jrheum.080262)

## Key Indexing Terms:

C-REACTIVE PROTEIN

SYSTEMIC LUPUS ERYTHEMATOSUS

GENES

The pathogenesis of systemic lupus erythematosus (SLE) is complex and multifactorial, involving interactions among multiple genes, hormones, and several environmental factors. Even though the etiopathogenesis of SLE remains elusive, it is believed that impaired handling of antigen–antibody complexes and subsequent tissue deposition leading to release of inflammatory mediators and an array of inflam-

matory cells can induce a broad spectrum of clinical manifestations<sup>1</sup>. Among a range of factors that may contribute to the pathophysiology of SLE, chronic inflammation is thought to play a pivotal role in the pathogenesis of SLE.

Family and twin studies suggest that genetic factors play a significant role in the predisposition to SLE<sup>2,3</sup>. The estimated heritability of SLE in Caucasians is 66%<sup>4</sup>. Recent genome-wide linkage analyses in multiplex SLE families have provided many chromosomal regions for exploration of disease-predisposing genes, including a region on the q-arm of chromosome 1<sup>5</sup>. The gene coding for C-reactive protein (CRP) is located at 1q23, which falls within the 1q23-43 region thought to harbor a susceptibility gene for SLE in multiple independent genome scans of both mice and humans<sup>6-10</sup>. The unique position of the *CRP* gene makes it a logical positional candidate gene to investigate as a susceptibility locus for SLE.

*CRP* is also a functional candidate gene based on the physiological activity of its products. CRP is an important liver-derived acute-phase protein that can increase up to 1000-fold in serum as a response to diverse stimuli such as infection or injury<sup>11</sup>. CRP has been shown to bind chromatin<sup>12</sup>, histones<sup>13</sup>, and apoptotic cells<sup>14</sup>. These unique

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characteristics of CRP are thought to contribute to its ability to modify the autoimmune disease phenotype by promoting the removal of necrotic and apoptotic cells and recruiting complement and Fc $\gamma$ R-mediated effector pathways<sup>15</sup>. In the host, the increased clearance of apoptotic cells and their derived nuclear contents by phagocytic cells via CRP opsonization may prevent the development of potential nuclear antigen-specific autoimmune responses<sup>14,16</sup>. Recent *in vivo* studies have shown that lupus-prone BW mice carrying the *CRP* transgene had reduced proteinuria, lived longer than non-transgenic BW, and had delayed accumulation of IgM and IgG in their renal glomeruli<sup>17</sup>. Injecting CRP to another lupus-strain mouse, NZB/NZW, also delayed the onset of high-grade proteinuria and prolonged survival<sup>18</sup>. CRP's autoimmunity prevention ability may come from its ability to prevent activation of autoreactive B cells by promoting clearance of autoantigens to non-antigen-presenting sites<sup>15</sup>.

Several studies have shown that CRP concentrations in patients with SLE are abnormally elevated in both the absence and presence of infection<sup>19-23</sup>. The value of using CRP to monitor SLE disease activity has remained controversial given the inconsistent correlation between circulating CRP and disease activity from numerous studies<sup>24-28</sup>. The abnormal elevation pattern of CRP in patients with SLE provided the first clinical clue that variation in the *CRP* may contribute to the pathogenesis of SLE. With CRP's unquestionable tie to inflammation, association with atherogenesis, its unique ability to modify the disease phenotypes of SLE, and its status as a positional candidate gene, *CRP* serves as a promising susceptibility gene for SLE.

Russell, *et al*<sup>29</sup> found basal levels of CRP to be influenced independently by 2 *CRP* polymorphisms (+838 and +2043), and the latter was also associated with SLE and antinuclear autoantibody production. They hypothesized that defective disposal of potentially immunogenic material, indicated by low basal CRP levels, may be a contributory factor in lupus pathogenesis. In our study, we examined 5 tagSNP (single-nucleotide polymorphisms), including +838 and +2043 both individually and as haplotypes, to investigate the associations of *CRP* with SLE risk and serum CRP levels in patients with SLE. We hypothesized that the variation in the *CRP* gene may contribute to the genetic susceptibility of SLE and may affect CRP levels in patients with SLE.

## MATERIALS AND METHODS

**Subjects.** A total of 337 Caucasian women with SLE and 448 healthy female controls were included in our study. All patients were 18 years of age or older (mean age 43  $\pm$  11 yrs, 40.3% postmenopausal, disease duration 10.13  $\pm$  7.13 yrs) and were recruited from the Pittsburgh Lupus Registry. All subjects met the 1982 and 1997 American College of Rheumatology (ACR) criteria for definite (n = 324) or probable (n = 13) SLE<sup>30,31</sup> at the time of recruitment.

Participating subjects in our study have been seen either at the University of Pittsburgh Medical Center or by practicing rheumatologists in

the Pittsburgh metropolitan area. The diagnosis of SLE was confirmed by a rheumatologist at the University of Pittsburgh (SM) prior to entry into the study. Since these patients are not exclusively from a tertiary referral center, they represent a spectrum of SLE that may be more reflective of a population-based sample.

Controls were race, sex, and geographically matched and recruited from the Central Bank of Pittsburgh, and had no apparent history of SLE (mean age 45  $\pm$  13 yrs, 100% Caucasian, 100% women). The study was approved by the University of Pittsburgh Institutional Review Board, and all subjects provided written informed consent.

**SLE clinical and laboratory characteristics.** A subset of SLE cases (n = 237, mean age 44.26  $\pm$  10.9 yrs, 40% postmenopausal) participating in a cardiovascular disease study in SLE had high-sensitivity CRP data for the current genetic association study<sup>32</sup>. None of the 237 SLE cases with CRP measurement had any evidence of infection at the time of the study CRP level (logCRP range -1.6 to 4.4).

CRP was measured using high-sensitivity enzyme linked immunosorbent assay, as described<sup>33,34</sup>. SLE disease activity and cumulative damage were measured by the same physician (SM) in all patients, using the Systemic Lupus Activity Measure (SLAM)<sup>35</sup> and the Systemic Lupus International Collaborating Clinics (SLICC) damage index<sup>36</sup>, respectively. Renal disease among patients with SLE was defined using the ACR criteria, which require either (1) renal biopsy showing lupus nephritis, or (2) persistent proteinuria > 0.5 g per day or > 3+ if quantification is not performed, or (3) evidence of cellular casts in the urine. Central nervous involvement (CNS) and arthritis among patients with SLE were defined by the ACR criteria including history of seizure or psychosis due to SLE for the former. Two hundred ninety-five patients (87.2%) had arthritis, 88 (26.1%) had a diagnosis of SLE-renal disease, and 30 (8.9%) had CNS involvement. Patients' mean SLICC score at the time of recruitment was 1.42 (SD 1.76) and mean SLAM score was 6.29 (SD 3.73). Additional measurements included anti-double stranded DNA, antiphospholipid antibodies, and serum C3 and C4.

**TagSNP selection and genotyping.** Five informative tagSNP were selected from a total of 31 known SNP in the SeattleSNPs Program for Genomic Applications website (<http://pga.gs.washington.edu/education.html>). SNP -861 and -390 are located in the promoter region, SNP +90 is located in the intron/exon boundary, +838 is a synonymous SNP present within exon 2, and +2043 maps in the 3'-untranslated region. We have designated our SNP based on their position relative to the ATG codon of the *CRP* translation site in the FASTA database. For clarification, reference numbers from the NCBI Entrez SNP database are provided for each of our 5 SNP: -861 is rs3093059, -390 is rs3091244, +90 is rs1417938, +838 is rs1800947, and +2043 is rs1205.

Genotyping for +838 and +2043 was obtained using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). SNP -861 was genotyped using pyrosequencing assays. SNP -390 and +90 were genotyped using TaqMan assays. TaqMan assays required a higher concentration of DNA that was not available in all subjects at the time of the experiment. Therefore genotyping was performed on 275 cases and 375 controls for the -390 and +90 SNP. Our genotyping success rates on all SNP ranged from 89% to 99% in the total sample. Ten percent of samples were re-genotyped (a second time) and the concordance rate was > 99%.

**Statistical analyses.** Allele frequencies were calculated by the allele counting method. Goodness of fit to Hardy-Weinberg expected proportions was examined by chi-squared test. The pairwise linkage disequilibrium (LD) between markers was estimated using the D' method<sup>37</sup>. The differences in genotype frequencies between cases and controls were tested by Fisher's exact test. Common haplotype frequency was estimated using the expectation-maximization algorithm in the EH software program<sup>38</sup> in both cases and controls. After detecting a significant association between *CRP* genetic variation and SLE risk, we performed followup tests to assess possible associations between *CRP* genetic variation and SLE clinical characteristics using either analysis of variance (ANOVA) for quantitative clinical

characteristics of SLE (C3, C4, SLAM, and SLICC) or logistic regression analysis for categorical variables (renal disease, joint inflammation, CNS involvement, and antiphospholipid antibodies). Covariates adjusted for in the models included age, body mass index (BMI), and smoking.

To assess the association between *CRP* genetic variation and serum CRP in subjects with SLE, CRP values were log-transformed to reduce non-normality. The mean log-transformed CRP (logCRP) levels between different genotype groups were compared using ANOVA and adjusted for the effects of age, BMI, and smoking. We also conducted haplotype association tests with logCRP using the haplo.stats package for R<sup>39</sup>, with age, BMI, and smoking included as covariates. Haplo.stats tests association by means of a generalized linear-regression framework that incorporates haplotype phase uncertainty by inferring a probability matrix of haplotype likelihoods for each individual (derived by use of the EH haplotype-inference algorithm) rather than by assignment of a most likely haplotype. All computations were performed using R version 2.1.2.

## RESULTS

**Association of *CRP* SNP with SLE risk.** Of the total 785 subjects (337 cases and 448 controls) genotyped for 5 *CRP* SNP, we repeated genotyping on 10% of the subjects for each SNP a second time, and had > 99% concordance rate in all SNP. No statistically significant deviations from Hardy–Weinberg equilibrium were found in any of the SNP. Table 1 presents the genotype and allele frequencies in our cases and controls for the 5 *CRP* SNP examined. Genotype and allele frequencies were not significantly different between cases and controls (using the p value of 0.05) in any of the 5 individual SNP examined.

**Association of *CRP* haplotype with SLE risk.** We conducted pairwise LD analysis using 4 SNP (excluding the triallelic SNP –390), and found different patterns of LD association in cases versus controls. In cases, with the exception of the –861/+90 and +90/+838 pairs, all SNP pairs were in signif-

icant LD. Among controls, all pairs were in significant LD except for the –861/+838 pair (Table 2). Because SNP haplotype may be more informative analyses of multiple SNP and the LD pattern may differ between cases and controls, we assessed the distribution of *CRP* haplotypes between cases and controls. All SNP (–861, –390, +90, +838, +2043) were included in our global haplotype analysis using 222 cases and 313 controls for which 100% genotyping data were available (Table 3). A total of 8 haplotypes were observed at a frequency of 2% or greater from either case or control groups. Even accounting for 2 tests (the logistic regression and haplotype analyses) of the relationship between the CRP SNP and case/control status, the overall haplotype distribution was significantly different between cases and controls (chi-square = 138.86, p < 0.001; Table 3). Haplotype 5 appears to be the most pronounced risk haplo-

Table 2. Pairwise linkage disequilibrium between CRP SNP.

	Pairwise Linkage Disequilibrium, SLE			D' (p)
	+90	+838	+2043	
–861	0.018 (0.945)	0.993 (0.037)	0.880 (< 0.001)	
+90		0.066 (0.511)	0.234 (0.016)	
+838			0.846 (< 0.001)	
	Pairwise Linkage Disequilibrium, Controls			D' (p)
	+90	+838	+2043	
–861	0.996 (< 0.001)	0.482 (0.319)	0.996 (< 0.001)	
+90		0.687 (0.002)	0.952 (< 0.001)	
+838			0.753 (< 0.001)	

CRP: C-reactive protein; SNP: single-nucleotide polymorphisms.

Table 1. Genotype and allele frequencies of CRP SNP.

SNP	Genotype	SLE Cases n (%)	Controls n (%)	p	Allele	SLE Cases n (%)	Controls n (%)	p		
–861 (rs3093059)	TT	287 (85.93)	388 (86.8)	0.813	T	621 (93)	834 (93.3)	0.802		
	TC	47 (14.07)	58 (12.98)		C	47 (7)	60 (6.7)			
	CC	0 (0)	1 (0.22)							
–390 (rs3091244)	CC	92 (38)	135 (41)	0.849	C	289 (59)	416 (61)	0.565		
	CT	88 (36)	118 (36)		T	161 (33)	196 (31)		0.451	
	TT	29 (12)	30 (9)		A	36 (7)	52 (8)			0.789
	CA	17 (7)	28 (8)							
	TA	15 (6)	18 (5)							
AA	2 (1)	3 (1)								
+90 (rs1417938)	AA	117 (48.75)	159 (47.89)	0.973	A	335 (69.8)	461 (69.4)	0.895		
	AT	101 (42.08)	143 (43.07)		T	145 (30.2)	203 (30.6)			
	TT	22 (9.17)	30 (9.04)							
+838 (rs1800947)	GG	283 (83.98)	395 (88.17)	0.125	G	619 (91.8)	840 (93.8)	0.14		
	GC	53 (15.73)	50 (11.16)		C	55 (8.2)	56 (6.3)			
	CC	1 (0.3)	3 (0.67)							
+2043 (rs1205)	GG	142 (42.51)	207 (46.31)	0.538	G	441 (66)	607 (67.9)	0.434		
	GA	157 (47.01)	193 (43.18)		A	227 (34)	287 (32.1)			
	AA	35 (10.48)	47 (10.51)							

CRP: C-reactive protein; SNP: single-nucleotide polymorphisms; SLE: systemic lupus erythematosus.

Table 3. CRP haplotype case-control comparison.

Haplotype	-861 (T>C)	-390 (C>T>A)	+90 (A>T)	+838 (G>C)	+2043 (G>A)	SLE Frequency (n = 222)	Control Frequency (n = 313)	Frequency Difference
H1	T	C	A	G	G	0.330	0.304	0.026
H2	T	C	A	G	A	0.183	0.259	-0.076
H3	T	C	A	C	A	0.047	0.050	-0.004
H4	T	T	T	G	G	0.201	0.286	-0.085
H5	T	T	T	G	A	0.061	0.002	0.058
H6	T	A	A	G	G	0.038	0.011	0.027
H7	C	C	A	G	G	0.032	0.004	0.028
H8	C	A	A	G	G	0.007	0.061	-0.054

Overall  $p < 0.001$ , chi-square = 138.86.

type for SLE, while haplotypes 2, 4, and 8 seem to convey protection against SLE. However, since no single allele at any locus defined and was restricted to a given risk or protective overall haplotype, no specific haplotype-tagging SNP could be identified to account for the significant overall haplotype associations.

*Association of CRP SNP with SLE clinical characteristics.* Because we detected a significant association between CRP SNP haplotypes and SLE, we performed followup analyses to determine if the CRP SNP were associated with specific SLE characteristics. These tests were done in a subgroup of 237 patients with SLE on whom we have clinical phenotype data, and we performed either ANOVA for quantitative clinical characteristics of SLE or logistic regression analysis for categorical variables, adjusting for the effects of age, BMI, CRP levels, and smoking when appropriate. Individuals with +838 GC genotype also exhibited nominally significantly higher C4 levels compared to GG individuals ( $23.46 \pm 0.54$  vs  $20.67 \pm 0.49$ ;  $p = 0.033$ ). No significant associations were observed between any of the individual SNP and SLAM, SLICC, C3, creatinine, renal disease, arthritis, and antiphospholipid antibodies (data not shown).

*CRP SNP associations with serum CRP concentrations.* We performed both single-site and haplotype analyses to assess the association between the 5 CRP SNP and log-transformed serum CRP levels (logCRP) in a subgroup of patients with SLE ( $n = 237$ ) in which CRP levels were available. After performing 5 tests of the single-site analyses, minor alleles of 2 SNP revealed significant associations with increased logCRP in patients with SLE (+90,  $p = 0.0032$ ; -390,  $p = 0.012$ ), even at the conservative Bonferroni level of significance ( $p = 0.01$ ). Homozygotes of the less common allele (T) at +90 had the highest logCRP level ( $1.544 \pm 0.271$ ) compared to homozygotes of the wild-type allele ( $0.639 \pm 0.120$ ) and the heterozygotes ( $0.623 \pm 0.117$ ). Mean logCRP levels were significantly higher in homozygotes of T allele at the triallelic promoter SNP -390 ( $1.305 \pm 0.259$ ) and heterozygotes with an A allele (CA) ( $1.356 \pm 0.286$ ) when compared to homozygotes of the wild-type (CC) ( $0.519 \pm 0.134$ ). SNP -861 and the 2 SNP (+838,

+2043) that were found to be associated with decreased CRP levels by Russell, *et al*<sup>29</sup> did not show a statistically significant effect on circulating CRP levels in this cohort (Table 4).

*CRP haplotype association with serum CRP levels.* Given the significant individual effects of SNP -390 and +90 on CRP levels and the LD between these SNP, and the observation that +90 is in high LD with -861, whereas -861 is in high LD with +838 and +2043 in cases, we performed 3-SNP haplotype analysis consisting of these 3 potentially functional SNP to evaluate the significance of the CRP promoter region on CRP levels. 3-SNP haplotypes were inferred using the haplo.glm function in the haplo.stats package in R. Haplotype -861C/-390T/+90T (H3 in Table 5) was associated with an increase of 1.171 logCRP units compared to the reference haplotype ( $p = 0.0161$ ), and is consistent with the results in Table 4 (the individual SNP

Table 4. Association of CRP polymorphisms and mean logCRP level ( $\pm$  SE).

SNP	Genotype	n (%)	Mean $\pm$ SE	p
-861* (rs3093059)	TT	191 (84.14)	0.718 $\pm$ 0.073	0.16
	TC	36 (15.86)	0.977 $\pm$ 0.184	
	CC	0 (0)	—	
-390* (rs3091244)	CC	50 (34.48)	0.519 $\pm$ 0.134	<b>0.012</b>
	CT	55 (37.93)	0.589 $\pm$ 0.121	
	TT	19 (13.10)	1.305 $\pm$ 0.259	
	CA	11 (7.59)	1.356 $\pm$ 0.286	
	TA	8 (5.52)	0.696 $\pm$ 0.351	
	AA	2 (1.38)	0.515 $\pm$ 0.596	
+90* (rs1417938)	AA	66 (45.83)	0.639 $\pm$ 0.120	<b>0.0032</b>
	AT	63 (43.75)	0.623 $\pm$ 0.117	
	TT	15 (10.42)	1.544 $\pm$ 0.271	
+838* (rs1800947)	GG	193 (83.91)	0.703 $\pm$ 0.075	0.373
	GC	37 (16.09)	0.869 $\pm$ 0.161	
	CC	0 (0)	—	
+2043* (rs1205)	GG	100 (43.67)	0.845 $\pm$ 0.112	0.207
	GA	107 (46.72)	0.605 $\pm$ 0.092	
	AA	22 (9.61)	0.857 $\pm$ 0.213	

\* Mean logCRP level ( $\pm$  SE) - Adjusted for age, body mass index, and smoking. CRP: C-reactive protein; SNP: single-nucleotide polymorphisms.

Table 5. Association of CRP promoter haplotype with serum logCRP levels in SLE.

	3-SNP Promoter Haplotype			Haplotype Frequency	Coefficient	SE	t	p
	-861 (T>C)	-390 (C>T>A)	+90 (A>T)					
Intercept	—	—	—	—	-1.651	0.364	-4.536	<b>0.000</b>
Age	—	—	—	—	0.014	0.007	2.064	<b>0.040</b>
BMI	—	—	—	—	0.048	0.010	4.887	<b>0.000</b>
Smoke	—	—	—	—	0.394	0.137	2.878	<b>0.004</b>
H2	C	C	A	0.050	0.028	0.273	0.103	0.918
H3	C	T	T	0.019	1.171	0.483	2.425	<b>0.016</b>
H4	T	A	A	0.074	0.263	0.246	1.072	0.285
H5	T	T	A	0.024	-0.158	0.384	-0.411	0.682
H6	T	T	T	0.301	0.293	0.143	2.042	<b>0.042</b>
H_other*	*	*	*	0.009	0.648	0.682	0.950	0.343
H1 = referent	T	C	A	0.522	Referent	—	—	—

t statistics and p values were calculated from coefficients and standard errors (SE) within the best-fit multivariate model by the haplo.glm function in the haplo.stats R package. \* Haplotypes with frequency < 2% were pooled as "H\_other." CRP: C-reactive protein; SLE: systemic lupus erythematosus.

associations). Haplotype -861C/-390T/+90T was also associated with an increase of logCRP by 0.2928 (p = 0.0423) (H6 in Table 5).

## DISCUSSION

We examined the association of *CRP* tagSNP in relation to SLE risk and CRP concentrations in patients with SLE. Individually, none of the examined SNP showed significant association with SLE risk. Russell, *et al*<sup>29</sup> reported that the minor (A) allele of +2043 was associated with SLE risk; but this association is not confirmed in our sample. However, in contrast to the single-site analysis, the *CRP* haplotype analyses yielded significant associations with SLE risk. The global 5-site *CRP* haplotype distribution was significantly different (nominal p < 0.001) between cases and controls, even after conservative Bonferroni adjustment for multiple comparisons (critical p value = 0.05/5 tests = 0.01). Further inspection of the haplotype results indicated that no single haplotype-tagging SNP explained the significant haplotype association with SLE risk, consistent with the individual SNP results.

The observed significant haplotype association in the absence of individual SNP association may be explained in several ways. First, the use of multilocus analyses in the SNP setting should improve the information content of genomic regions<sup>40</sup> and may also identify effects from multiple polymorphisms (vs single tagSNP analysis) as well as subtle interaction effects (epistasis)<sup>41</sup> within the given haplotype block. Second, haplotypes can mark unique chromosomal segments that harbor susceptibility alleles, even if the LD patterns differ between study populations. Given that LD patterns are likely to differ between groups due to population history and/or genetic admixture, our finding that *CRP* haplotype frequencies differ between SLE cases and controls is consistent with previous results from Russell, *et al*<sup>29</sup>. Both studies indicate that *CRP* polymorphisms are

associated with SLE risk; however, neither study has identified strong evidence of a specific susceptibility allele. Even though the individual tagSNP approach has been the gold standard for association studies for many years, it requires that a SNP be in strong LD with a causal polymorphism that has a measurable effect. Given the polygenic and multifactorial character of SLE pathogenesis, the haplotype approach may be more useful in detecting genotype-phenotype associations in comparison to the individual SNP approach, especially if multiple and/or uncommon variants are associated with the SLE risk. As noted in Materials and Methods, there are 31 SNP reported in the SeattleSNPs Program for Genomic Applications, of which 5 were sufficiently polymorphic for analysis purposes in our study sample.

Genetic variation at the *CRP* locus could influence SLE risk via its effect on CRP levels, and Russell, *et al*<sup>29</sup> reported a significant association between CRP levels and genotypes at SNP +2043 and +838. We did not observe significant association between CRP levels and genotypes at these 2 SNP, but we did observe significant association between genotypes at a promoter SNP (SNP -390, nominal p = 0.01) and a SNP at an intron/exon boundary (SNP +90, nominal p = 0.003), even after Bonferroni adjustment for 5 tests. In fact, our result that the -390T allele is associated with increased CRP levels directly supports previous observations that T allele forms an E-Box binding site that is involved in transcription binding<sup>42</sup>. Additional *in silico* prediction analysis and *in vitro* data confirmed that haplotypes containing the -390T allele increase reporter gene activity significantly<sup>43</sup>. Among our SLE cases, homozygotes for the -390T allele had a 2-fold increase in logCRP (1.544 ± 0.271) compared to homozygotes of the wild-type allele (0.639 ± 0.120) and heterozygotes (0.623 ± 0.117; p = 0.0032; Table 4).

Polymorphisms located in gene promoters may play a role in gene function by altering transcription factor identi-

fication and binding, which in turn can influence gene expression and affect biological pathways. Similarly, SNP at the intron/exon boundary may result in alternative splicing and affect gene function. Indeed, these relationships between CRP levels at these 2 individual SNP have also been reported in healthy, non-SLE populations<sup>43-45</sup>. In addition, our haplotype analyses of the 2 promoter and 1 splice site SNP (Table 5) further indicate that all 3 SNP contribute to increased CRP levels.

Russell, *et al*<sup>29</sup> reported significant association between SNP +838 and +2043 and decreased CRP level in a British SLE cohort. Similarly, a more recent study by Miller, *et al*<sup>44</sup> reported the same association of these 2 SNP in 3 large cohorts of the healthy general population. However, we did not observe the same association between SNP +838 and +2043 and decreased CRP in our 273 patients with SLE. The lack of association in these women with SLE may be attributed to the limited sample size of the minor allele carriers in our study, or it may be confounded by the effects from antiinflammatory medications patients with SLE take on a regular basis, like corticosteroids.

Although our association results between *CRP* genotypes and CRP concentrations are consistent with some previous reports, they differ from others. Determining the true association between genetic variation and CRP levels is inherently difficult due to the complex mechanism of CRP production, which is activated by cytokines interleukin 6 (IL-6) and IL-1 and influenced by multiple other genes and environmental factors<sup>46</sup>. The difficulty is compounded in an SLE cohort because not only are the inflammatory cytokines increased in patients with SLE<sup>47</sup>, but the strong correlation between CRP and IL-6 levels in healthy subjects may be absent in SLE<sup>48</sup>. SLE is a chronic inflammatory disease with abnormal expression of *CRP* during both the presence and absence of acute infections. Multiple studies have also found inconsistent correlations between CRP levels and SLE disease activity, indicating that the mechanism influencing *CRP* expression in individuals with SLE may differ from that in the general population. Our data showed that even though the exon 2 and 3' region SNP did not correlate with significantly decreased CRP levels in our patients with SLE, as others have found, individual SNP and haplotype in the promoter region revealed associations with increased basal CRP levels as shown in the general population. Our findings emphasize the important functional role *CRP* promoter polymorphisms may play in their expression even in patients with a chronic inflammatory disease.

Russell's family-based study proposed that low basal levels of CRP may predispose to antinuclear autoantibody production, which in turn contributes to the development of human lupus<sup>29</sup>. Our results show that individually, certain SNP are correlated with CRP levels, but their association with SLE risk was not significant. Although we did not find strong evidence that any of the individual *CRP* SNP influ-

ence CRP level and thereby predict SLE risk, the significant global haplotype results suggest that variation in the *CRP* gene modifies SLE risk via as yet unidentified mechanisms. Our individual SNP results, coupled with promoter haplotype results, confirm the previous studies done in the general population showing that *CRP* promoter variants have a significant influence on CRP levels in patients with SLE. The lack of association with SNP +838 and +2043 and decreased CRP levels in our patients may result from years of SLE insult (mean disease duration 10.13 yrs) from the chronic inflammatory state.

It remains a possibility that *CRP* itself does not directly contribute to SLE susceptibility, rather one or more as yet unidentified SLE susceptibility alleles in nearby loci may be in strong LD with one or more of the *CRP* SNP we examined. Two potential SLE susceptibility genes that also mapped to 1q23, *FcγRIIA* and *FcγRIIIA*, encode for low-affinity receptors for IgG. Recent metaanalyses revealed that the *FcγRIIA-R/H131* polymorphism was associated with a 1.3-fold greater risk of development of lupus, and that the *FcγRIIIA-V/F158* polymorphism conferred 1.4-fold risk for developing lupus nephritis<sup>49</sup>. The interaction of IgG Fc receptors containing an activation motif (ITAM) with immune complexes and cytotoxic autoantibodies can initiate an inflammatory response leading to tissue damage<sup>50</sup>. It has also been demonstrated that *FcγRIIA-R/H131*, working in conjunction with CRP, has the unique ability to alter the cytokine profile of the host<sup>51</sup> by mediating phagocytosis<sup>52</sup>, and contributing to the impaired removal of circulating immune complexes<sup>53</sup>, resulting in the antibody-triggered inflammation and disease pathogenesis of SLE and nephritis. Given the overlapping chromosomal position of the human *CRP*, *FcγRIIA*, and *FcγRIIIA* genes and their unique ability to modify SLE phenotype when working together, it is likely that genetic interaction between these 3 loci (epistasis) may modify SLE susceptibility.

Our study is consistent with some previous studies showing that genetic variation in *CRP* influences risk of SLE and levels of CRP in patients with SLE, although the same genetic variation did not influence CRP levels and SLE risk in our study, and some of our single SNP association results differ from those of other studies. These results may indicate that the SNP may not act via level alone, but exert their effects via different kinds of activity or interactions with other proteins. Further, complex diseases and traits (such as SLE and CRP levels) are likely to be influenced by multiple genes, each exerting effects in small to modest range<sup>54</sup>. A limitation of our study is the relatively small sample size, which reduces our ability to detect genes with small effects as well as effects of gene-by-gene and gene-by-environment interactions. In addition, as circulating CRP is a sensitive acute-phase protein that could easily be fluctuated by multiple factors, longitudinal studies of CRP and SLE would be useful. Future work is necessary, perhaps using murine

models<sup>17,18</sup>, to determine the true mechanism underlying the associations between *CRP* genetic variation and SLE risk. Such information will further our understanding of SLE etiology and may have direct clinical relevance.

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