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 inflammatory cells can induce a broad spectrum of clinical manifestations[1]. 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[2,3]. The estimated heritability of SLE in Caucasians is 66%[4]. 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[5]. 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[6-10]. 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[11]. CRP has been shown to bind chromatin[12], histones[13], and apoptotic cells[14]. 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 FcγR-mediated effector pathways[15]. 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[14,16]. 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[17]. Injecting CRP to another lupus-strain mouse, NZB/NZW, also delayed the onset of high-grade proteinuria and prolonged survival[18]. CRP's autoimmunity prevention ability may come from its ability to prevent activation of autoreactive B cells by promoting clearance of antoantigens to non-antigen-presenting sites[15].

Several studies have shown that CRP concentrations in patients with SLE are abnormally elevated in both the absence and presence of infection[19-23]. 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[24-28]. 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*[29] 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 ± 11 yrs, 40.3% postmenopausal, disease duration 10.13 ± 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[30,31] 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 ± 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 ± 10.9 yrs, 40% postmenopausal) participating in a cardiovascular disease study in SLE had high-sensitivity CRP data for the current genetic association study[32]. 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 immunobsorbent assay, as described[33,34]. SLE disease activity and cumulative damage were measured by the same physician (SM) in all patients, using the Systemic Lupus Activity Measure (SLAM)[35] and the Systemic Lupus International Collaborating Clinics (SLICC) damage index[36], 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[37]. 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[38] 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[39], 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 significant 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			
	+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)	D' (p)
+838			0.846 (< 0.001)	
	Pairwise Linkage Disequilibrium, Controls			
	+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)	D' (p)
+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	TT	287 (85.93)	388 (86.8)	0.813	T	621 (93)	834 (93.3)	0.802
(rs3093059)	TC	47 (14.07)	58 (12.98)		C	47 (7)	60 (6.7)	
	CC	0 (0)	1 (0.22)					
–390	CC	92 (38)	135 (41)	0.849	C	289 (59)	416 (61)	0.565
(rs3091244)	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	AA	117 (48.75)	159 (47.89)	0.973	A	335 (69.8)	461 (69.4)	0.895
(rs1417938)	AT	101 (42.08)	143 (43.07)		T	145 (30.2)	203 (30.6)	
	TT	22 (9.17)	30 (9.04)					
+838	GG	283 (83.98)	395 (88.17)	0.125	G	619 (91.8)	840 (93.8)	0.14
(rs1800947)	GC	53 (15.73)	50 (11.16)		C	55 (8.2)	56 (6.3)	
	CC	1 (0.3)	3 (0.67)					
+2043	GG	142 (42.51)	207 (46.31)	0.538	G	441 (66)	607 (67.9)	0.434
(rs1205)	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 ± 0.54 vs 20.67 ± 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 ± 0.271) compared to homozygotes of the wild-type allele (0.639 ± 0.120) and the heterozygotes (0.623 ± 0.117). Mean logCRP levels were significantly higher in homozygotes of T allele at the triallelic promoter SNP –390 (1.305 ± 0.259) and heterozygotes with an A allele (CA) (1.356 ± 0.286) when compared to homozygotes of the wild-type (CC) (0.519 ± 0.134). SNP –861 and the 2 SNP (+838, +2043) that were found to be associated with decreased CRP levels by Russell, *et al*[29] 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 (± SE).

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

* Mean logCRP level (± 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							
	–861 (T>C)	–390 (C>T>A)	+90 (A>T)	Haplotype Frequency	Coefficient	SE	t	p
Intercept	—	—	—	—	–1.651	0.364	–4.536	**0.000**
Age	—	—	—	—	0.014	0.007	2.064	**0.040**
BMI	—	—	—	—	0.048	0.010	4.887	**0.000**
Smoke	—	—	—	—	0.394	0.137	2.878	**0.004**
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	**0.016**
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	**0.042**
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*[29] 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[40] and may also identify effects from multiple polymorphisms (vs single tagSNP analysis) as well as subtle interaction effects (epistasis)[41] 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*[29]. 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*[29] 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[42]. Additional *in silico* prediction analysis and *in vitro* data confirmed that haplotypes containing the –390T allele increase reporter gene activity significantly[43]. 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[43-45]. 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*[29] 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*[44] 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[46]. The difficulty is compounded in an SLE cohort because not only are the inflammatory cytokines increased in patients with SLE[47], but the strong correlation between CRP and IL-6 levels in healthy subjects may be absent in SLE[48]. 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[29]. 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 influence 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[49]. 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[50]. 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[51] by mediating phagocytosis[52], and contributing to the impaired removal of circulating immune complexes[53], 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[54]. 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[17,18], 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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REFERENCES

1. Kyttaris VC, Katsiari CG, Juang Y-T, Tsokos GC. New insights into the pathogenesis of systemic lupus erythematosus. Curr Rheumatol Rep 2005;7:469-75.
2. Block SR, Winfield JB, Lockshin MD, D'Angelo WA, Christian CL. Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. Am J Med 1975;59:533-52.
3. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, et al; Grupo Latinoamericano de Estudio del Lupus Eritematoso. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. Arthritis Rheum 2005;52:1138-47.
4. Lawrence JS, Martins CL, Drake GL. A family survey of lupus erythematosus. 1. Heritability. J Rheumatol 1987;14:913-21.
5. Tsao BP. Update on human systemic lupus erythematosus genetics. Curr Opin Rheumatol 2004;16:513-21.
6. Johanneson B, Lima G, von Salome J, Alarcon-Segovia D, Alarcon-Riquelme ME: Collaborative Group on the Genetics of SLE and Sjögren's Syndrome. A major susceptibility locus for systemic lupus erythemathosus maps to chromosome 1q31. Am J Hum Genet 2002;71:1060-71.
7. Mohan C, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis. Sle1 on murine chromosome 1 leads to a selective loss of tolerance to h2a/h2b/DNA subnucleosomes. J Clin Invest 1998;101:1362-72.
8. Moser KL, Neas BR, Salmon JE, et al. Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in African American pedigrees. Proc Natl Acad Sci USA 1998;95:14869-74.
9. Tsao BP, Cantor RM, Grossman JM, et al. Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to systemic lupus erythematosus. Arthritis Rheum 2002;46:2928-36.
10. Scofield RH, Bruner GR, Kelly JA, et al. Thrombocytopenia identifies a severe familial phenotype of systemic lupus erythematosus and reveals genetic linkages at 1q22 and 11p13. Blood 2003;101:992-7.
11. Kushner I. The acute phase response: An overview. Methods Enzymol 1988;163:373-83.
12. Robey FA, Jones KD, Tanaka T, Liu TY. Binding of C-reactive protein to chromatin and nucleosome core particles. A possible physiological role of C-reactive protein. J Biol Chem 1984;259:7311-6.
13. Du Clos TW, Zlock LT, Rubin RL. Analysis of the binding of C-reactive protein to histones and chromatin. J Immunol 1988;141:4266-70.
14. Gershov D, Kim S, Brot N, Elkon KB. C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: Implications for systemic autoimmunity. J Exp Med 2000;192:1353-64.
15. Szalai AJ. C-reactive protein (CRP) and autoimmune disease: Facts and conjectures. Clin Dev Immunol 2004;11:221-6.
16. Kravitz MS, Pitashny M, Shoenfeld Y. Protective molecules — C-reactive protein (CRP), serum amyloid p (SAP), pentraxin3 (PTX3), mannose-binding lectin (MBL), and apolipoprotein a1 (Apo a1), and their autoantibodies: Prevalence and clinical significance in autoimmunity. J Clin Immunol 2005;25:582-91.
17. Szalai AJ, Weaver CT, McCrory MA, et al. Delayed lupus onset in (NZB x NZW)f1 mice expressing a human C-reactive protein transgene. Arthritis Rheum 2003;48:1602-11.
18. Rodriguez W, Mold C, Marnell LL, et al. Prevention and reversal of nephritis in mrl/lpr mice with a single injection of C-reactive protein. Arthritis Rheum 2006;54:325-35.
19. Maury CP, Wegelius O. Clinical value of serum amyloid and C-reactive protein measurements in secondary amyloidosis. Int J Tissue React 1985;7:405-7.
20. Becker GJ, Waldburger M, Hughes GR, Pepys MB. Value of serum C-reactive protein measurement in the investigation of fever in systemic lupus erythematosus. Ann Rheum Dis 1980;39:50-2.
21. Morrow WJ, Isenberg DA, Parry HF, Snaith ML. C-reactive protein in sera from patients with systemic lupus erythematosus. J Rheumatol 1981;8:599-604.
22. Eberhard OK, Haubitz M, Brunkhorst FM, Kliem V, Koch KM, Brunkhorst R. Usefulness of procalcitonin for differentiation between activity of systemic autoimmune disease (systemic lupus erythematosus/systemic antineutrophil cytoplasmic antibody-associated vasculitis) and invasive bacterial infection. Arthritis Rheum 1997;40:1250-6.
23. Roy S, Tan KT. Pyrexia and normal C-reactive protein (CRP) in patients with systemic lupus erythematosus: Always consider the possibility of infection in febrile patients with systemic lupus erythematosus regardless of CRP levels. Rheumatology Oxford 2001;40:349-50.
24. Inoue T, Kanayama Y, Katoh N, et al. Significance of C-reactive protein elevation in the pretreatment stage of systemic lupus erythematosus. Scand J Rheumatol 1981;10:222-4.
25. Linares LF, Gomez-Reino JJ, Carreira PE, Morillas L, Ibero I. C-reactive protein (CRP) levels in systemic lupus erythematosus (SLE). Clin Rheumatol 1986;5:66-9.
26. Hind CR, Ng SC, Feng PH, Pepys MB. Serum C-reactive protein measurement in the detection of intercurrent infection in oriental patients with systemic lupus erythematosus. Ann Rheum Dis 1985;44:260-1.
27. Bertouch JV, Roberts-Thompson PJ, Feng PH, Bradley J. C-reactive protein and serological indices of disease activity in systemic lupus erythematosus. Ann Rheum Dis 1983;42:655-8.
28. Barnes EV, Narain S, Naranjo A, et al. High sensitivity C-reactive protein in systemic lupus erythematosus: Relation to disease activity, clinical presentation and implications for cardiovascular risk. Lupus 2005;14:576-82.
29. Russell AI, Cunninghame Graham DS, Shepherd C, et al. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. Hum Mol Genet 2004;13:137-47.
30. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
31. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
32. Selzer F, Sutton-Tyrrell K, Fitzgerald S, Tracy R, Kuller L, Manzi S. Vascular stiffness in women with systemic lupus erythematosus. Hypertension 2001;37:1075-82.
33. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: Implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52-8.
34. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the cardiovascular

health study. Clin Chem 1995;41:264-70.
35. Liang MH, Socher SA, Larson MG, Schur PH. Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. Arthritis Rheum 1989;32:1107-18.
36. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363-9.
37. Lewontin RC. The interaction of selection and linkage. II. Optimum models. Genetics 1964;50:757-82.
38. Terwilliger JD, Ott J. Handbook of human genetic linkage. Baltimore: Johns Hopkins University Press; 1994.
39. Lake SL, Lyon H, Tantisira K, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;55:56-65.
40. Ott J, Rabinowitz D. The effect of marker heterozygosity on the power to detect linkage disequilibrium. Genetics 1997;147:927-30.
41. Davidson S. Research suggests importance of haplotypes over SNPs. Nat Biotechnol 2000;18:1134-5.
42. Szalai AJ, Wu J, Lange EM, 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;83:440-7.
43. Carlson CS, Aldred SF, Lee PK, 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.
44. Miller DT, Zee RYL, Suk Danik J, et al. Association of common CRP gene variants with CRP levels and cardiovascular events. Ann Hum Genet 2005;69:623-38.
45. Lange LA, Carlson CS, Hindorff LA, et al. Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. JAMA 2006;296:2703-11.
46. Wessel J, Moratorio G, Rao F, et al. C reactive protein, an "intermediate phenotype" for inflammation: Human twin studies reveal heritability, association with blood pressure and the metabolic syndrome, and the influence of common polymorphism at catecholaminergic/ß-adrenergic pathway loci. J Hypertension 2007;25:329-43.
47. Asanuma Y, Chung CP, Oeser A, et al. Increased concentration of proatherogenic inflammatory cytokines in systemic lupus erythematosus: Relationship to cardiovascular risk factors. J Rheumatol 2006;33:539-45.
48. Williams RC Jr, Harmon ME, Burlingame R, Du Clos TW. Studies of serum C-reactive protein in systemic lupus erythematosus. J Rheumatol 2005;32:454-61.
49. Karassa FB, Trikalinos TA, Ioannidis JPA. The role of FcγRIIA and IIIA polymorphisms in autoimmune diseases. Biomed Pharmacother 2004;58:286-91.
50. Clynes R, Calvani N, Croker BP, Richards HB. Modulation of the immune response in pristane-induced lupus by expression of activation and inhibitory Fc receptors. Clin Exp Immunol 2005;141:230-7.
51. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J Immunol 2002;169:7019-25.
52. Bodman-Smith KB, Gregory RE, Harrison PT, Raynes JG. FcγRIIA expression with FcγRI results in C-reactive protein- and IgG-mediated phagocytosis. J Leukoc Biol 2004;75:1029-35.
53. Zuniga R, Markowitz GS, Arkachaisri T, Imperatore EA, D'Agati VD, Salmon JE. Identification of IgG subclasses and C-reactive protein in lupus nephritis: The relationship between the composition of immune deposits and Fcγ receptor type IIA alleles. Arthritis Rheum 2003;48:460-70.
54. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.