Monocyte Chemoattractant Protein-1: Plasma Concentrations and A(–2518)G Promoter Polymorphism of Its Gene in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To determine (1) whether the A(–2518)G polymorphism of *CCL-2*, the gene encoding monocyte chemoattractant protein-1 (MCP-1), is associated with disease, MCP-1 concentration, nephritis, or coronary artery calcification (CAC) in systemic lupus erythematosus (SLE); and (2) whether MCP-1 and homocysteine (Hcy) concentrations are correlated.

Methods. Statistical tests were applied to determine the relationships between *CCL-2* A(–2518)G genotypes, plasma MCP-1 concentrations, and clinical variables in Caucasian and African American patients with SLE and controls.

Results. The *CCL-2* (–2518)G allele was not significantly associated with SLE in the whole study sample ($p = 0.07$). Among Caucasians, but not African Americans, G allele carriers had significantly increased risk of SLE (OR 4.2, 95% CI 1.8–9.6, $p < 0.0001$). Genotype was not associated with nephritis, CAC, or MCP-1 concentrations when all patients or all controls were considered; however, among recently diagnosed patients, G allele carriers had significantly higher MCP-1 concentrations than AA homozygotes ($p = 0.02$). SLE patients had higher MCP-1 concentrations than controls ($p < 0.0001$), African American patients had higher concentrations than Caucasian patients ($p = 0.006$), and patients with nephritis had higher concentrations than those without nephritis ($p = 0.02$). Although not associated with CAC, MCP-1 concentrations were significantly positively correlated with Hcy.

Conclusion. *CCL-2* A(–2518)G genotype is a significant risk factor for SLE among Caucasians but not African Americans, suggesting that genetically mandated differences in MCP-1 expression contribute to SLE etiology in the former. The positive correlation between MCP-1 and Hcy concentrations is consistent with the hypothesis that active inflammation and hyperhomocysteinemia are etiologically linked. (J Rheumatol 2007;34:740–6)

Key Indexing Terms:
MONOCYTE CHEMOATTRACTANT PROTEIN-1 INFLAMMATORY DISEASE
CCL-2 NEPHRITIS HOMOCYSTEINE SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is an autoimmune disease with a strong inflammatory component. It affects women much more frequently than men, and is particularly prevalent among African Americans, suggesting that there are sex and race-specific etiological factors.

Renal disease (lupus nephritis) is a major cause of morbidity and mortality in SLE. Accumulation of macrophages and leukocytes in the glomeruli and interstitial spaces of the kidney is a significant contributor to nephritis, and the proinflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) is important in the recruitment of these cells[1]. Some studies suggest that urinary MCP-1 in patients with nephritis correlates with disease severity[2] and flares[3]. In a mouse model of SLE, MCP-1 antagonists prevented the development of nephritis, suggesting that this chemokine plays a central role in inflammation mediated kidney damage[4,5].

Increased MCP-1 expression may also be a contributor to the development of premature atherosclerotic cardiovascular disease (ASCVD) in patients with SLE. Potentially, MCP-1 plays an important role in the development of atherosclerosis by recruiting inflammatory cells across the vessel wall early in lesion formation, and it is expressed in established lesions

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at high concentrations, thus facilitating lesion growth. High MCP-1 concentrations have been positively associated with both coronary artery calcification (CAC) and intima-media thickness[6,7]. MCP-1 concentrations are elevated in SLE patients relative to controls, although concentrations in plasma do not always correlate well with disease activity[2].

Homocysteine (Hcy), which is an established risk marker for ASCVD in the general population, is also elevated (i.e., hyperhomocysteinemia) in patients with SLE[8]. In a recent case-control study we found that hyperhomocysteinemia in SLE was highly associated with CAC, as determined by electron beam computed tomography (EBCT)[8]. CAC appears in the very early stages of ASCVD and progresses with the disease; further, CAC scores determined by EBCT indicate total atherosclerotic burden and are predictive of future cardiac events. The mechanism underlying the association between Hcy and CAC has not been determined. However, studies in cultured cells and animal models suggest that hyperhomocysteinemia may induce MCP-1, in addition to having other proinflammatory effects[9-13], and that this may constitute an etiologic link between hyperhomocysteinemia and CAC.

A functional polymorphism, A(–2518)G, in the promoter region of *CCL-2*, the gene that encodes MCP-1, modifies the degree to which MCP-1 expression is induced by proinflammatory stimuli[14]. Relative to the (–2518)A allele, the (–2518)G allele is associated with increased interleukin 1 mediated MCP-1 transcription and higher circulating concentrations of MCP-1 protein[14,15]. Reports on the *CCL-2* polymorphism in relation to SLE have been contradictory. The AG/GG genotypes have been reported to confer increased risk of SLE in a North American sample[16], but not in Spanish or Korean samples[17-19]. However, the G allele has been associated variously with nephritis in a North American SLE sample[16], cutaneous vasculitis in a Spanish SLE sample[17], nephritis in Chinese children with SLE[20], and arthritis in Chinese adults with SLE[21].

We hypothesized that the *CCL-2* (–2518)G allele would be associated with increased MCP-1 plasma concentrations and increased risk for both nephritis and ASCVD in patients with SLE. Because elevated Hcy may be etiologically linked to MCP-1 concentration, we also hypothesized that MCP-1 and Hcy plasma concentrations would be positively correlated. To test these hypotheses, we determined the *CCL-2* A(–2518)G genotypes and MCP-1 plasma concentrations in a sample of SLE patients and controls for whom Hcy, nephritis, and CAC data were already available. The prevalence of SLE is particularly high among African Americans, suggesting the existence of race-specific genetic risk factors. Therefore all statistical tests were carried out both before and after stratification of the study sample by race.

MATERIALS AND METHODS

Consecutive, nonpregnant, female patients with SLE over age 18 years (n = 152) and controls (n = 132) were enrolled. SLE patients were required to fulfill at least 4 of the American College of Rheumatology revised criteria for the classification of SLE[22]. Controls, recruited from University of Pennsylvania clinics, were each matched to a patient for age, sex, and race. The study was approved by the Institutional Review Board of the University of Pennsylvania, and written informed consent was obtained from each participant.

Clinical assessments. Medical history and physical examination data were collected for all subjects at their single study visit. Treatment status for the following medications was also recorded: methotrexate, azathioprine, cyclophosphamide, mycophenolate mofetil, prednisone, hydroxychloroquine, angiotensin-converting enzyme inhibitors, beta blockers, statins, antidepressants, and aspirin. SLE disease activity was measured by the SLE Disease Activity Index (SLEDAI)[23], and organ damage was evaluated with the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index (SLICC/ACR-DI)[24]. All subjects also underwent EBCT as described[8,25].

Laboratory assessments. Fasting blood and urine samples were collected from subjects at their single study visit and frozen at –80°C until the time of evaluation. Laboratory assessments included the following: complete blood count, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), total cholesterol, triglycerides, fibrinogen, creatinine, Westergren erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (CRP), dilute Russell viper venom test, anticardiolipin IgG and IgM, anti-β_2-glycoprotein I IgG and IgM, anti-dsDNA, and complement C3 and C4. Antibody concentrations were determined by ELISA. Glomerular filtration rate (GFR) was calculated for each subject using the Modification of Diet in Renal Disease equation[26]. Standard urinalysis was conducted. In those patients identified with nephritis, all but 2 patients had the clinical diagnosis confirmed by renal biopsy.

A separate blood sample was drawn for the measurement of Hcy concentrations. Total plasma Hcy concentrations were determined using the AxSYM homocysteine via fluorescence polarization immunoassay (Abbot Laboratories, Abbot Park, IL, USA).

CCL-2 genotyping. Genomic DNA was extracted from whole blood using Generation Capture Columns (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. *CCL-2* A(–2518)G genotypes were determined using a Taqman real-time polymerase chain reaction (PCR) assay on a DNA Engine Opticon 2 continuous fluorescence detection system (Bio-Rad, Hercules, CA, USA). PCR amplification was performed using 3 μl of genomic DNA, 0.2 μM forward (5'-TTC TTG ACA GAG CAG AAG TGG-3') and reverse (5'-GCC TTT GCA TAT ATC AGA CAG TA-3') primers, 1 pmol A allele probe (6-FAM-5'-AFA CAG CTA TCA CTT-3'-MGBNFQ) and 2 pmol G allele probe (5'-VIC-AGA CAG CTG TCA CTT TC-3'-MGBNFQ) in Taqman master mix (Applied Biosystems, Foster City, CA, USA). Probes were custom synthesized by Applied Biosystems. Each PCR was performed in a 20 μl reaction with an initial incubation at 50°C for 2 min, then 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s and extension at 57°C for 1 min. Dual fluorescence was detected after each extension step. Genotype interpretations were performed using Opticon Monitor Analysis software, version 2.02 (Bio-Rad).

MCP-1 plasma concentrations. Plasma MCP-1 concentrations were determined in serum samples by ELISA (Peprotech, Rocky Hill, NJ, USA) according to the manufacturer's instructions.

Statistical analysis. Four subjects with MCP-1 concentrations > 1000 pg/ml (3 patients and one control) were excluded from the analyses on the basis that they were atypical extreme outliers. In addition, subjects lacking both MCP-1 plasma concentration and *CCL-2* A(–2518)G genotype data were excluded. The analysis dataset after exclusion of these subjects comprised 147 patients and 131 controls. Of these, 144 patients and 130 controls had *CCL-2* genotype data, 144 patients and 130 controls had MCP-1 concentration data, and 141 patients and 129 controls had data for both.

Geometric means and standard deviations were calculated for the following variables, which were log-transformed prior to analysis to correct for a rightward skew: Hcy, MCP-1, VLDL, HDL, LDL, triglycerides, CRP, dsDNA, anticardiolipin IgG, anticardiolipin IgM, anti-β_2-glycoprotein IgG,

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anti-ß$_2$-glycoprotein IgM, C3, C4, and fibrinogen. Median and interquartile ranges were calculated for disease duration. Means and standard deviations were calculated for all other continuous variables. CAC also showed a pronounced rightward skew; however, as this variable was considered inappropriate for log-transformation due to the number of 0 values, nonparametric tests were used on the untransformed CAC values as described below. Genotype frequency deviations from Hardy-Weinberg equilibrium were assessed by chi-square analysis. Medication use frequencies for patients versus controls, *CCL-2* A(–2518)G genotype frequencies for patients versus controls, and the proportion of patients with nephritis or being treated with prednisone among Caucasian and African American patients were compared by Fisher's exact test. Pairwise comparisons for log-transformed and normally distributed variables by disease status (patient or control), by subset, and by *CCL-2* genotype were performed by t test. Multivariate comparisons of the effects of race, nephritis status, and prednisone treatment status, without and with genotype, were carried out by factorial ANOVA. The Wilcoxon rank-sum test was used to evaluate the association between genotype and CAC and to compare disease duration between the African American and Caucasian subsets. Associations between MCP-1 concentration or *CCL-2* genotype with SLICC-DI and SLEDAI were assessed by Kruskal-Wallace test and chi-square analysis, respectively. Correlations between log-transformed MCP-1 concentrations and continuous variables were assessed both by visual inspection of scatter plots and by using the Pearson correlation coefficient, except in the case of CAC, which was correlated with MCP-1 concentrations using the nonparametric Kendall tau-b correlation coefficient.

To determine significant predictors of MCP-1 concentrations, a series of regression models were constructed in patients after exclusion of subjects who were not either Caucasian or African American. Race, nephritis, prednisone treatment status, genotype, and all variables that significantly correlated with MCP-1 concentrations, as well as 2-way multiplicative interactions between variables retained in the model as significant main effects and race, prednisone treatment status, or nephritis status, were considered.

RESULTS

Sample characteristics. The demographic and main clinical characteristics of patients and controls are summarized in Table 1. A fuller description of the demographic, clinical, and

Table 1. Sample characteristics.

Characteristic	Patients	Controls	p
No.	147	131	ND
Age, yrs, mean ± SD	43.8 ± 11.6	43.6 ± 10.7	0.9
Race†, % (n)			0.26
Caucasian	36.7 (54)	42.0 (55)	
African American	53.7 (79)	53.4 (70)	
Asian	4.8 (7)	3.8 (5)	
Hispanic	3.4 (5)	0.0 (0)	
Other	1.4 (2)	0.8 (1)	
BMI, mg/kg^2, mean ± SD (n)	28.6 ± 7.2 (103)	29.8 ± 7.4 (131)	0.22
Homocysteine, μM, mean ± SD (n)	11.3 ± 1.4 (147)	9.8 (1.3) (131)	0.0001
MCP-1, pg/ml, mean ± SD (n)	44.1 ± 2.1 (144)	24.7 ± 1.5 (130)	< 0.0001
CCL-2 A (-2518) G genotype, % (n)			
AA	61.1 (88)	71.5 (93)	0.10
AG	33.3 (48)	22.3 (29)	
GG	5.6 (8)	6.2 (8)	
CCL-2 G allele carriers, % (n)			
Caucasian	53.7 (29)	21.8 (12)	0.0008
African American	22.4 (17)	29.0 (20)	0.45
CAC, median (interquartile range)	0 (0–7.6)	0 (0–0)	0.002
Vascular events*, % (n)	28.08 (41)	4.58 (6)	< 0.0001
Myocardial infarction	2.7 (4)	1.5 (2)	0.49
DVT	16.6 (24)	0.0 (0)	< 0.0001
Pulmonary embolism	1.4 (2)	1.5 (2)	0.91
Stroke	11.7 (17)	2.3 (3)	0.0027
VLDL, mg/dl, mean ± SD (n)	17.1 ± 2.5 (141)	11.8 ± 2.3 (130)	0.0008
LDL, mg/dl, mean ± SD (n)	100.2 ± 1.5 (147)	117.1 ± 1.4 (131)	0.0005
HDL, mg/dl, mean ± SD (n)	53.9 ± 1.4 (147)	59.2 ± 1.3 (131)	0.006
TG, mg/dl, mean ± SD (n)	95.7 ± 1.8 (146)	75.7 ± 1.6 (131)	0.0004
Disease duration, yrs, mean ± SD (n)	11.2 ± 8.6 (147)	NA	ND
SLICC-DI, median (IQR) (n)	2 (1–3) (146)	NA	ND
SLEDAI, median (IQR) (n)	4 (2–7) (146)	NA	ND
Current renal disease, % (n)**	35.4 (52)	0	< 0.0001
GFR, ml/min/1.73 m^2, mean ± SD (n)	87.6 ± 32.8 (147)	93.6 ± 21.1 (131)	0.07
ESR, mm/h, mean ± SD (n)	31.8 ± 27.0 (146)	ND	ND
hsCRP, mg/l, mean ± SD (n)	2.9 ± 4.1 (142)	2.1 ± 4.1 (130)	0.05
ds-DNA, IU/ml, mean ± SD (n)	42.9 ± 4.3 (130)	ND	ND

† p value calculated for race distribution within each sample and not for individual races. * History of at least one event. ** Nephritis or renal failure. CAC: coronary calcification (raw Agatston's score), BMI: body mass index, MCP-1: monocyte chemoattractant protein-1, DVT: deep vein thrombosis, TG: triglycerides, GFR: glomerular filtration rate, ND: not done, NA: not applicable, IQR: interquartile range.

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biochemical data for this sample has been reported[8]. Patients had an average age of 43.8 ± 11.6 years, an average disease duration of 11.2 ± 8.6 years, a median SLICC/ACR-DI of 2, and a median SLEDAI of 4. The patient group comprised 36.7% Caucasian, 53.7% African American, and 9.6% Asian, Hispanic or other. The control group had similar age and race distributions. Patients and controls differed significantly in Hcy, MCP-1, CAC, VLDL, LDL, HDL, triglycerides, and CRP concentrations, and in percentage with nephritis, or a history of at least one vascular event [pulmonary embolism (PE), deep vein thrombosis (DVT), stroke, or myocardial infarction]. There was also a trend toward lower GFR in patients relative to controls (87.6 vs 93.6; p = 0.07). Significantly more patients than controls were treated with prednisone, hydroxychloroquine, methotrexate, mycophenolate mofetil, azathioprine, cyclophosphamide, beta-blockers, and antidepressants (data not shown). However, treatment with statins and aspirin did not differ significantly between patients and controls (data not shown).

CCL-2 A(–2518)G genotype and SLE. Patients and controls were genotyped for the *CCL-2* A(–2518)G promoter polymorphism. Genotype frequencies (Table 1) were in Hardy-Weinberg equilibrium among controls, and in the control Caucasian and African American subsets. When distributions were compared between patients and controls, there was a trend toward overrepresentation of G alleles in patients (p = 0.13) and a stronger trend toward G allele carriers (i.e., those with either the GA or GG genotype) in patients (p = 0.07). Therefore, for all subsequent genetic analyses, the AG and GG genotypes were pooled.

Patients and controls were next stratified by race, and the distribution of *CCL-2* genotypes was compared (Table 1). In Caucasians, the G allele carriers were significantly overrepresented in patients relative to controls (53.7% and 21.8%, respectively; p = 0.0008), whereas in African Americans, there was no significant difference in AG/GG genotype frequency between patients and controls (22.4% and 29.0%, respectively; p = 0.45). This suggests that Caucasian G allele carriers are 4-fold more likely to have SLE than those with the AA genotype (OR 4.2, 95% CI 1.8–9.6, p < 0.0001).

When the patient sample was stratified by the presence of nephritis, there was no significant difference in genotype frequencies between those with and those without this condition (38.4% and 33.2% AG/GG, respectively; p = 0.52). When this analysis was restricted to Caucasian patients, the AG/GG genotypes were overrepresented among those with nephritis (67% vs 50%); however, this difference did not reach statistical significance (p = 0.35), possibly because of the small numbers involved (only 12 Caucasian patients had nephritis). Among African American patients with and without nephritis, the AG/GG frequency was similar.

When the patient sample was stratified by prednisone treatment at baseline, there was also no significant difference in genotype frequencies between treated and untreated patients, either in the entire sample (37.5% and 41.3% AG/GG, respectively; p = 0.73), or within the African American (31.3% and 32.1% AG/GG; p = 1.0) or Caucasian (39.1% and 41.9% AG/GG; p = 1.0) subsets.

MCP-1 plasma concentrations. Plasma MCP-1 concentrations were measured, and tests of association with SLE, nephritis, prednisone treatment, and genotype were performed. MCP-1 concentrations were significantly higher in patients than in controls (means of 44.3 and 24.8 pg/ml, respectively; p < 0.0001; Table 2), in patients with nephritis compared with patients without nephritis (means of 54.1 and 39.7 pg/ml; p = 0.02; Table 2), and in patients treated with and not treated with prednisone (means of 50.2 and 36.4 pg/ml, respectively; p = 0.006; Table 2). African American patients had higher concentrations than Caucasian patients (means of 48.9 and 34.8 pg/ml; p = 0.006). This difference is consistent with the observations that significantly more of the former had nephritis (46.8% vs 22.2%; p = 0.006) and were undergoing current prednisone treatment (62.0% vs 42.6%; p = 0.03). When the effects of race, nephritis, and prednisone treatment were compared by factorial ANOVA, nephritis and prednisone were significant as main effects, but race was not, and none of these factors interacted significantly with each other. The back-transformed least-square mean MCP-1 concentrations from the ANOVA are presented in Table 3.

MCP-1 concentrations were not associated with *CCL-2* A(–2518)G genotype in controls, whether stratified by race or not (data not shown). This result was not surprising, given the presumptive absence of overt inflammatory stimuli in controls. Further, as shown in Table 4, MCP-1 concentrations were not significantly associated with genotype in patients or controls as a whole, in the Caucasian or African American patient subsets, in patients with or without nephritis, or in patients treated with prednisone or not. Since carrying the (–2518)G allele was associated with increased risk for SLE itself, we hypothesized that AG/GG genotypes might be associated with higher MCP-1 concentrations in the early stages of disease, in which case the initial transcriptional advantage conferred by the G allele would be superseded by other regulatory mechanisms driving MCP-1 expression in established SLE. To test this hypothesis, we examined MCP-1 concentrations in patients with recently diagnosed disease (i.e., disease duration ≤ 1 year). Although the number of such patients was small (n = 11), MCP-1 concentrations were significantly higher in G allele carriers than in AA homozygotes in this category (70.91 and 19.00 pg/ml, respectively; p = 0.03). As expected, there was no such association between MCP-1 concentration and genotype in patients with longer disease duration (Table 4). Importantly, there was no significant difference in median disease duration between African Americans and Caucasians (medians of 11 and 8 yrs, respectively; p = 0.39), and there was only a weak trend toward overall correlation between disease duration and MCP-1 concentrations (Kendall's tau-b correlation coefficient = 0.086, p = 0.13).

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Table 2. Plasma MCP-1 concentrations by disease status.

Subset	MCP-1 Concentration, mean ± SD, pg/ml (n) Patients	Controls	p
None	44.26 ± 2.12 (144)	24.78 1.54 (130)	< 0.0001
With nephritis	54.05 ± 1.86 (50)	NA	ND
Without nephritis	39.65 ± 2.20 (94)	24.29 ± 1.52 (127)	< 0.0001
Current prednisone treatment	50.23 ± 2.29 (79)	23.28 ± 1.52 (2)	0.20
No current prednisone treatment	36.38 ± 1.71 (64)	24.66 ± 1.54 (126)	< 0.0001
Caucasian	34.81 ± 1.88 (53)	24.78 ± 1.51 (55)	< 0.0001
African American	48.91 ± 2.08 (77)	24.78 ± 1.58 (69)	0.001

ND: Not done. NA: not applicable.

Disease duration, nephritis status, prednisone treatment status, and genotype distribution for the African American and Caucasian subsets are shown in Table 5. When the contributions of these 4 factors were assessed by factorial ANOVA, once again only nephritis status and prednisone treatment status were significant, both as main effects, and no 2-way interactions were significant (data not shown).

MCP-1 and cardiovascular disease and markers of cardiovascular disease. The relationships of *CCL-2* genotype or MCP-1 concentrations with CAC, history of at least one vascular event (i.e., DVT, PE, myocardial infarction, stroke), and several risk factors for ASCVD (i.e., Hcy, VLDL, LDL, HDL, triglycerides, CRP) were assessed in patients with SLE. *CCL-2* genotype was not significantly associated with CAC, prior vascular event, or any individual cardiovascular risk factor.

Table 3. SLE patients: adjusted least-square mean MCP-1 concentration (pg/ml) from factorial ANOVA.

Subset	Mean	n	p*
With nephritis	49.50	47	0.03
Without nephritis	37.41	83	
Current prednisone treatment	48.47	70	0.05
No current prednisone treatment	38.21	60	
Caucasian	38.55	53	0.08
African American	48.04	77	

* Bonferroni corrected.

However, MCP-1 concentrations were significantly positively correlated with concentrations of Hcy, triglycerides, and CRP, and negatively correlated with HDL; no correlations with any of the other ASCVD risk factors were observed (Table 6). Since Hcy has been associated with the induction of MCP-1 expression in cultured cells, we examined the relationship between MCP-1 and Hcy in more detail. MCP-1 concentrations for SLE patients with a Hcy concentration in the highest quartile were nearly twice those for patients with Hcy concentration in the lowest quartile (mean 61.2 ± 2.77 vs 37.4 ± 1.9 pg/ml, respectively; p = 0.01; n = 38 and 36, respectively).

MCP-1 and other laboratory variables. Tests for association between MCP-1 concentrations and other laboratory variables in patients were applied as described in the statistical methods section. MCP-1 concentrations were positively correlated with ESR, anti-dsDNA, and anticardiolipin IgM (but not anticardiolipin IgG), and negatively correlated with GFR (Table 6).

Linear regression models. To determine whether Hcy remained a significant predictor of MCP-1 concentrations when other significant correlates, in particular prednisone treatment or nonspecific measures of inflammation, were considered, a series of linear regression models were fitted to the data as described above. A significant interaction between prednisone treatment status and ESR was dropped from the final model because it did not substantially increase the percentage of MCP-1 variation explained by the model, and no other interactions were significant. GFR did not contribute to

Table 4. Effect of *CCL-2* A (-2518) G genotype on plasma MCP-1 concentrations.

Sample Subset	MCP-1 Concentration, mean ± SD, pg/ml (n) AA	AG/GG	p
All patients	42.92 ± 2.04 (86)	44.17 ± 2.23 (55)	0.85
All controls	24.06 ± 1.49 (92)	26.49 ± 1.65 (37)	0.25
Caucasian patients	36.91 ± 2.19 (24)	33.15 ± 1.62 (29)	0.56
African American patients	44.68 ± 1.89 (58)	60.62 ± 2.66 (16)	0.13
Patients with nephritis	52.76 ± 1.89 (31)	54.16 ± 1.81 (18)	0.89
Patients without nephritis	38.20 ± 2.08 (55)	40.00 ± 2.40 (37)	0.78
Patients treated with prednisone	48.46 ± 2.13 (48)	51.63 ± 2.58 (30)	0.75
Patients not treated with prednisone	32.51 ± 1.70 (37)	40.17 ± 1.63 (25)	0.12
Disease duration			
≤ 1 yr	19.00 ± 1.17 (4)	70.81 ± 3.37 (7)	0.03
2–5 yrs	46.09 ± 1.97 (10)	47.40 ± 2.94 (8)	0.92
> 5 yrs	44.05 ± 2.06 (76)	39.36 ± 1.75 (38)	0.43

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Table 5. Comparison of African American and Caucasian subsets of patients with SLE.

Variable	Subset	African Americans	Caucasians	p
Disease duration, yrs; median [IQR] (n)	None	11 [4–18] (79)	8 [4-17] (54)	0.39†
Genotype, % (n)				
AA		68.4 (52)	59.3 (32)	0.35††
AG/GG		31.6 (24)	41.7 (22)	
Nephritis status, % (n)				
With		46.8 (37)	22.2 (12)	0.006††
Without		53.2 (42)	77.8 (42)	
Prednisone, % (n)				
Currently treated		62.0 (49)	42.6 (30)	0.03††
Currently not treated		38.0 (30)	57.4 (31)	

† Wilcoxon rank-sum test. †† Fisher's exact test. IQR: interquartile range.

Table 6. MCP-1 concentration correlates in patients with SLE.

Variable	Pearson Correlation Coefficient	p
ESR	0.43	< 0.0001
hsCRP	0.38	< 0.0001
Anticardiolipin IgM	0.33	< 0.0001
Triglycerides	0.30	0.0003
ds-DNA	0.28	0.003
Homocysteine	0.24	0.004
HDL	–0.18	0.03
GFR	–0.17	0.05

HDL: high density lipoprotein, GFR: glomerular filtration rate.

the model and was not included. The final model included Hcy, anti-dsDNA, CRP, and ESR as main effects. These terms accounted for 40% of the variation in MCP-1 plasma concentrations (adjusted r^2). Parameters for this final model are given in Table 7. Only 31% of the variation could be explained by a regression model that included anti-dsDNA, CRP, and ESR, but not Hcy, as main effects, indicating that 9% of the variation in MCP-1 concentration in the SLE patients under test was attributable to Hcy.

DISCUSSION

We observed that the G allele of the *CCL-2* A(–2518)G promoter polymorphism confers a 4-fold increased risk of SLE in Caucasian women, but not African American women. Other studies that investigated this polymorphism in SLE have not stratified for race[16-21]. Plasma MCP-1 concentrations were significantly higher in patients than in controls, consistent with other reports[16-21]. Interestingly, G allele carriers who had been diagnosed within the previous year had higher MCP-1 concentrations than their AA homozygous peers. This difference was not observed in those with more lengthy disease. It is therefore possible that MCP-1 plays a critical role in initiating and sustaining inflammatory processes during the early phase of SLE, and that the *CCL-2* A(–2518)G polymorphism confers differential responsiveness to aspects of the underlying disease process at that time. Such differential responsiveness might subsequently be lost as inflammatory aspects of pathology become more complex in established SLE.

In other studies of SLE, the *CCL-2* (–2518)G allele has been associated with an increased risk of lupus nephritis[16,19,20]. In our SLE patient sample, however, we found no significant association of genotype with nephritis, either in the entire sample or after stratification by race. Nevertheless, although the G allele was not significantly associated with nephritis in our small subset of Caucasians, a much higher proportion of Caucasian patients with nephritis than without had the AG/GG genotypes. No such overrepresentation of the AG/GG genotypes was seen in African American patients with nephritis. A race-specific differential influence of the *CCL-2* A(–2518)G polymorphism on lupus nephritis would have important implications for both mechanistic studies of the etiology of kidney damage in SLE and the appropriate management of patients with particular genotypes. Therefore the contribution of the G allele to risk of nephritis in Caucasian patients needs to be assessed in a larger sample.

Consistent with our *a priori* hypothesis, MCP-1 concentrations were strongly positively correlated with Hcy concentrations in patients with SLE. This relationship was independent of nephritis status and GFR, and remained significant in a regression model after adjustment for the correlation between MCP-1 and markers of both inflammation (CRP, ESR) and disease activity (anti-dsDNA). Hcy explained 9% of the MCP-1 variation after adjustment for these other factors. Although we have previously established that elevated Hcy is also strongly correlated with CAC in this sample[8], and high MCP-1 concentration was correlated with other traditional risk factors for ASCVD (i.e., elevated triglycerides and low-

Table 7. Multivariate regression parameter estimates in patients with SLE.

Variable	Parameter Estimate	Standard Error	p*
Intercept (ß)	1.18	0.46	0.01
hsCRP	0.13	0.04	0.004
ESR	0.01	0.002	0.001
Homocysteine	0.69	0.18	0.0002
ds-DNA	0.15	0.04	0.0002

* Partial t test.

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ered HDL), we did not detect any relationship between MCP-1 concentration and CAC. Others have reported that MCP-1 concentrations show highly significant positive correlation with CAC in a North American sample without systemic disease, as well as correlations with CRP and traditional ASCVD risk factors[7]. However, for patients with SLE, in whom chronic systemic inflammation is a prominent clinical feature, markers of inflammation have not been shown to correlate with ASCVD. Our study therefore reinforces the view that sensitive inflammatory markers that are useful for detecting ASCVD in individuals without overt inflammatory disease are much less useful for detecting emerging cardiovascular comorbidity in inflammatory diseases such as SLE.

We observed that *CCL-2* A(–2518)G genotype is a significant risk factor for SLE among Caucasian, but not African American, women. This suggests that differential expression of MCP-1, mandated by CCL-2 genotype, influences etiology only in the former. However, neither genotype nor plasma MCP-1 concentrations were associated with CAC, a measure of early ASCVD, indicating that a high concentration of this potent proinflammatory chemokine is not a good marker of ASCVD risk in patients with established SLE. The positive correlation between Hcy and MCP-1 concentrations is consistent with the hypothesis that elevated Hcy *per se*, or perturbations in folate/Hcy metabolism that lead to hyperhomocysteinemia, contribute to the induction of MCP-1 expression. However, since we observed no correlation between elevated MCP-1 and CAC, it is unlikely that the association between Hcy and CAC previously observed in this SLE sample[8] was primarily mediated by a Hcy-dependent increase in MCP-1 concentrations.

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