Functional Polymorphisms of Folate-Metabolizing Enzymes in Relation to Homocysteine Concentrations in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To determine if functional polymorphisms of folate/homocysteine pathway enzymes are associated with homocysteine concentrations and/or coronary artery calcification (CAC) scores in patients with systemic lupus erythematosus (SLE) and controls.

Methods. We investigated 163 SLE patients and 160 controls. Functional polymorphisms in 6 genes in the folate/homocysteine pathway were genotyped: 5,10-methylenetetrahydrofolate reductase (*MTHFR*) 677C>T, *MTHFR* 1298A>C, cystathionine ß-synthase (*CBS*) 844ins68, methionine synthase (*MTR*) 2756A>G, methionine synthase reductase (*MTRR*) 66A>G, thymidylate synthase (*TYMS*) 1494del6, and dihydrofolate reductase (*DHFR*) c.86+60_78.

Results. Homocysteine levels were higher in African American SLE patients than Caucasian patients and African American controls. Genotype distributions were significantly different in African American and Caucasian controls for 6 of the 7 polymorphisms. Genotype distributions for each polymorphism did not differ significantly between SLE patients and controls even after stratification by race. Glomerular filtration rate was strongly negatively correlated to homocysteine levels, and was therefore adjusted for as a covariate in the models of the effects of the polymorphisms on homocysteine levels. In SLE patients none of the 7 polymorphisms was associated with homocysteine concentrations. In Caucasian controls only MTHFR 677C>T and 1298A>C showed effects on homocysteine similar to what would be expected from the literature. There were no genotypic associations with median CAC scores in SLE patients or controls with and without stratification by race.

Conclusion. Polymorphisms in folate/homocysteine metabolizing enzymes do not predict higher homocysteine levels or CAC scores in patients with SLE. (First Release Sept 1 2008; J Rheumatol 2008;35:2179–86; doi:10.3899/jrheum.080071)

Key Indexing Terms:
HOMOCYSTEINE GENETIC POLYMORPHISMS SYSTEMIC LUPUS ERYTHEMATOSUS ATHEROSCLEROSIS CORONARY ARTERY CALCIFICATION

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that occurs predominantly in women, and more commonly among African Americans[1]. Individuals with SLE have a greater risk of developing premature atherosclerotic cardiovascular disease (ASCVD) than the general population[2]. Subclinical ASCVD can be measured by electron-beam computed tomography (EBCT), which is used to detect coronary artery calcification (CAC). CAC scores are related to risk of future cardiovascular events[3].

Atherosclerosis is a complex process and high homocysteine concentration is one component of that process. High circulating homocysteine concentrations are known to be associated with an increased risk of ASCVD in the general population[4]. Folate/homocysteine metabolism is important for DNA synthesis and for generating 5-methyltetrahydrofolate, the source of methyl groups that are ultimately used in many methylation reactions (Figure 1). Homocysteine concentrations can be modified by several factors including dietary intake of folate and other B vitamins, lifestyle variables, and genetic polymorphisms in the enzymes of the folate/homocysteine pathway[5]. One of the most widely studied such enzymes is 5,10-methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20), which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that is in turn used in the remethylation of homocysteine to methion-

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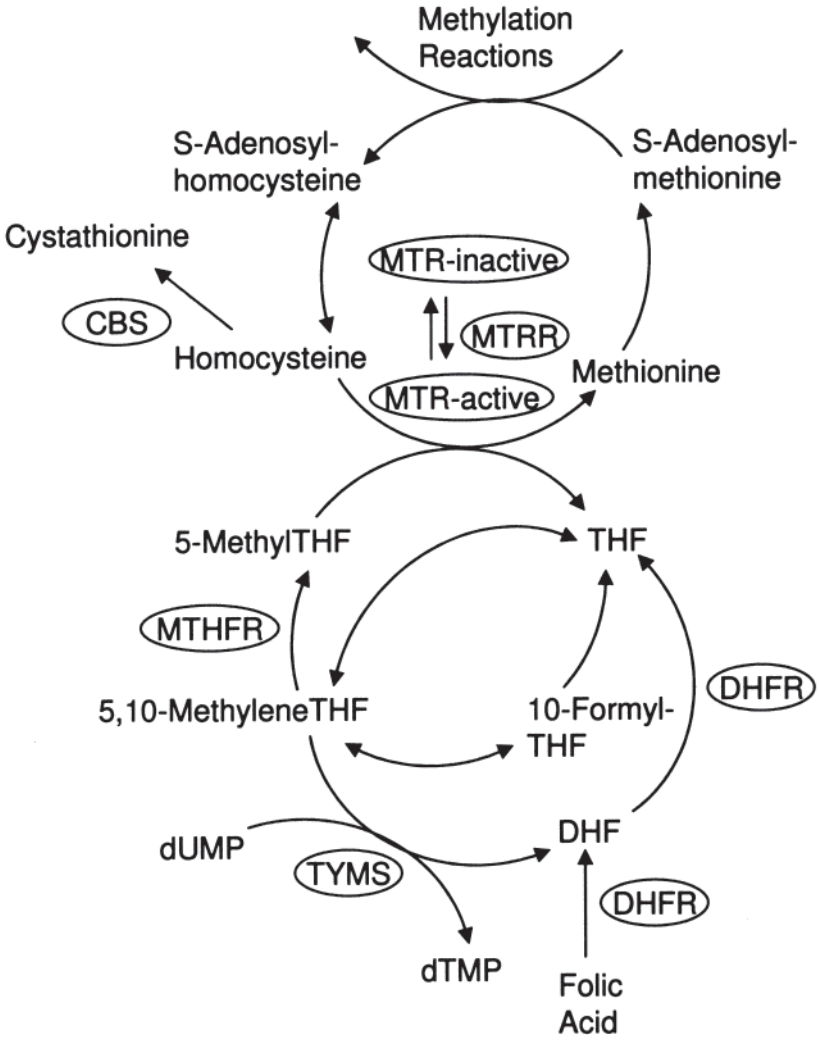

Figure 1. The folate/homocysteine pathway. CBS: cystathionine ß-synthase; DHF: dihydrofolate; DHFR: dihydrofolate reductase; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; MTHFR: 5,10-methylenetetrahydrofolate reductase; MTR: methionine synthase; MTRR: methionine synthase reductase; THF: tetrahydrofolate; TYMS: thymidylate synthase.

ine by methionine synthase (MTR; EC 2.1.1.13). The latter reaction requires methionine synthase reductase (MTRR; EC 2.1.1.135) to maintain the MTR cofactor cobalamin in its active form. The vitamin B_6-dependent enzyme cystathionine ß-synthase (CBS; EC 4.2.1.22) irreversibly metabolizes homocysteine to cystathionine in the initial step of the transsulfuration pathway. Thymidylate synthetase (TYMS; EC 2.1.1.45) uses 5,10-methylenetetrahydrofolate to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Dihydrofolate reductase (DHFR; EC 1.5.1.3) catalyzes the conversion of dihydrofolate to tetrahydrofolate and is also important for converting synthetic folic acid from vitamin supplements and fortified food into a biologically relevant derivative that can be used by cells. All these enzymes play important roles in maintaining a healthy cell phenotype, and dysfunction in any could potentially cause atherogenic changes. This is evidenced by patients with homocystinuria due to CBS deficiency that have premature atherosclerosis and thrombosis[6]. All these enzymes have common functional polymorphisms (*MTHFR* 677C>T and 1298A>C, *MTR* 2756A>G, *MTRR* 66A>G, CBS 844ins68, *TYMS* 1494del6, and *DHFR* c.86+60_78) that are known to affect homocysteine concentrations in some populations[7-16].

Patients with SLE have elevated homocysteine levels compared to matched controls[17-20], and high homocysteine in such patients is associated with the presence of CAC[19]. The extent, if any, to which polymorphisms in the above enzymes contribute to high homocysteine and CAC is unknown. We investigated to determine whether polymor-

phisms in key enzymes of the folate/homocysteine pathway are associated with increased homocysteine levels and/or CAC scores in patients with SLE.

MATERIALS AND METHODS

SLE and control subjects. Consecutive nonpregnant women over 18 years of age with SLE attending the University of Pennsylvania clinics and female controls matched for race and age (± 2 yrs) were invited to participate in the study; 163 patients and 160 controls were enrolled. SLE patients were required to fulfill at least 4 of the American College of Rheumatology revised criteria for the classification of SLE[21]. Controls were matched to patients with regard to key demographic features, and biochemical analyses indicated that they had similar traditional cardiovascular risk factor profiles but were otherwise healthy with no underlying inflammatory disease or exposure to corticosteroids. The study was approved by the University of Pennsylvania Institutional Review Board, and written informed consent was obtained from each participant.

Clinical assessments. A medical history was collected for all subjects. All participants had a physical examination and electrocardiography, gave a fasting blood sample, and underwent EBCT[19]. Clinical characteristics were as reported[19,22]. Framingham point scores were calculated based on a published method of scoring various cardiovascular risk factors[23]. Glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease equation[24].

Homocysteine measurement. Whole blood (5 ml) was drawn into EDTA and placed on ice until centrifuged at 2500 rpm for 5 min at room temperature. Plasma homocysteine concentrations were determined by fluorescence polarization immunoassay (AxSYM Homocysteine; Abbott Laboratories, Abbott Park, IL, USA). This assay was performed by the hospital's clinical laboratory, which used Abbott's homocysteine low and high controls daily and participated in CAP proficiency and linearity studies.

Genetic analysis. DNA was isolated using Generation Capture Column Kits (Gentra Systems, Minneapolis, MN, USA). *MTHFR* 677C>T (rs1801133) genotypes were analyzed using a heteroduplex generator method[25], and a portion of samples were repeated using the TaqMan assay. *MTHFR* 677C>T, *MTHFR* 1298A>C (rs1801131), *MTR* 2756A>G (rs1805087), and *MTRR* 66A>G (rs1801394) were genotyped by TaqMan real-time polymerase chain reaction (PCR) assays on a DNA Engine Opticon 2 continuous fluorescence detection system (Bio-Rad, Hercules, CA, USA). PCR amplifications were performed using 20 ng genomic DNA with forward and reverse primers, allele-specific probes (at concentrations listed in Table 1), and

Table 1. PCR primers, probes, concentrations, and conditions. Polymorphic site underlined in probe sequences.

Gene and Polymorphism (dbSNP RS no.)	Primers and Probes, 5'–3' Sequence	Concentration	Conditions
	TaqMan		
MTHFR 677C > T	F: GCA GGG AGC TTT GAG GCT GAC C	0.5 μM	92°C 30 s
(1801133)	R: TGG GGC AAG TGA TGC CCA TGT	0.5 μM	56°C 1 min
	*T: 6FAM-ATG AAA TCG A<u>C</u>T CCC GC-MGBNFQ	50 μM	50 cycles
	*C: VIC-ATG AAA TCG G<u>C</u>T CCC GC-MGBNFQ	100 μM	
MTHFR	F: GAG GAG CTG CTG AAG ATG T	0.5 μM	92°C 30 s
1298A > C	R: CGA GAG GTA AAG AAC GAA GA	0.5 μM	56°C 1 min
(1801131)	*C: 6FAM-AGA CAC TT<u>G</u> CTT CAC T-MGBNFQ	50 μM	50 cycles
	*A: VIC-CAA AGA CAC TT<u>T</u> CTT C-MGBNFQ	50 μM	
MTR 2756A > G	F: AGT GTT CCC AGC TGT TAG ATG A	0.5 μM	92°C 30 s
(1805087)	*R: TGT TTC TAC CAC TTA CCT TGA GAG ACT	0.5 μM	60°C 1 min
	*G: 6FAM-ACA GG<u>G</u> CCA TTA TG-MGBNFQ	50 μM	50 cycles
	*A: VIC-ATT AGA CAG G<u>A</u>C CAT TAT G-MGBNFQ	100 μM	
MTRR 66A > G	*F: CAT GCC TTG AAG TGA TGA GG	0.5 μM	92°C 30 s
(1801394)	*R: GAT CTG CAG AAA ATC CAT GTA CCA	0.5 μM	60°C 1 min
	*G: 6FAM-CTT GCT CAC A<u>C</u>A TTT-MGBNFQ	50 μM	50 cycles
	*A: VIC-TGC TCA CA<u>T</u> ATT TC-MGBNFQ	100 μM	
	Size Difference PCR		
CBS 844ins68	F: TAT TGG CCA CTC CCA TAA TAG A	0.4 μM	94°C 5 min
	R: CGG CTC TGC GAG GAT GGA CCC TT	0.4 μM	35 cycles of
			94°C 1 min
			55°C 1 min
			72°C 1 min
TYMS 1494de16	F: CAT GAT GTA GAG TGT GGT TAT G	0.4 μM	94°C 2 min
(16430)	R: GAA TGA ACA AAG CGT GGA	0.4 μM	35 cycles of
			94°C 30 s
			51°C 30 s
			72°C 30s then
			72°C 5 min
DHFR	F1: CCA CGG TCG GGG TAC CTG GG	0.4 μM	94°C 4 min
c.86+60_78	F2: ACG GTC GGG GTG GCC GAC TC	0.4 μM	35 cycles of
	R: AAA AGG GGA ATC CAG TCG G	0.8 μM	94°C 55s
			62°C 55 s
			72°C 55 s
			then 72°C 12 min

* Originated from SNP500Cancer website[26].

TaqMan Universal PCR MasterMix (Applied Biosystems, Foster City, CA, USA). Some primer and probe sequences were derived from the SNP500Cancer website as designated in Table 1[26]. MGB probes were custom-synthesized by Applied Biosystems. Each PCR was performed in 20 µl with an initial incubation at 50°C for 2 min, then 95°C for 10 min, followed by 50 cycles of denaturation and extension under the conditions listed in Table 1. Dual fluorescence was detected after each extension step. Genotype interpretations were performed using Opticon Monitor Analysis software, version 2.02 (Bio-Rad).

Size-difference PCR methods were used to genotype *CBS* 844ins68[25], *TYMS* 1494del6 (rs16430), and *DHFR* c.86+60_78[27]. PCR amplifications took place in 25 µl volumes that contained 50 ng genomic DNA, 0.4 µM of each forward and reverse primer (0.8 µM reverse primer for *DHFR* assay), 0.8 µM dNTPs, 10× PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$, and 1 U AmpliTaq DNA polymerase (Applied Biosystems). Cycling conditions are listed in Table 1. PCR products were separated on 3% agarose gels, run at 140 V for 45 min, and stained with ethidium bromide.

Statistical analysis. SAS version 9.1 was used for all statistical analysis with Type I error rate set to 0.05. Homocysteine was log-transformed to better approximate normality in all analyses. Hardy-Weinberg equilibrium for each of the genotypes was assessed by chi-square test. Differences in genotype frequency distributions between African American and Caucasian controls and between case and control groups were assessed by chi-square and Fisher's exact test. Correlations with log homocysteine were assessed by Pearson's correlation coefficients for age, GFR, and Framingham point scores. Student's t-test was used for assessment of smoking status and use of B_6, B_{12}, and folic acid on log homocysteine. General linear modeling was used to assess the effect of the above correlated variables on homocysteine. When race was used as a classification variable in the models, this included 127 Caucasian and 163 African American SLE cases and controls. Any variable that significantly contributed to the model was used as a covariate in assessment of the effect of genotype on log homocysteine as well as an interaction term with genotype to assess effect modification. Results of log homocysteine analyses were back-transformed to report results in original measurement units (µmol/l).

RESULTS

Sample characteristics. The mean age of SLE patients was 43.3 ± 11.0 years (Table 2). The race distribution was 50.3% African American, 39.3% Caucasian, 4.9% Asian, 4.3% Hispanic, and 1.2% other, with controls having a similar distribution. Median CAC scores were significantly higher in SLE patients than controls (p = 0.0003). Homocysteine concentrations were also higher in patients than controls (10.4 vs 9.2 µmol/l; p < 0.0001). African American SLE patients had higher homocysteine levels than African American controls (12.1 vs 9.7 µmol/l, p < 0.0001), while Caucasian SLE patients did not differ significantly from Caucasian controls (10.0 vs 9.0 µmol/l, p = 0.12). African American controls did not differ significantly from Caucasian controls in homocysteine concentrations (p = 0.21), but African American SLE patients had higher homocysteine levels than Caucasian patients (p = 0.0009). SLE patients did not differ significantly from controls in terms of Framingham point scores or GFR.

Genotype frequency distributions of African American and Caucasian controls. SLE patients and controls were genotyped for 7 polymorphisms in 6 enzymes in the folate/homocysteine pathway (*MTHFR* 677C>T and 1298A>C, *MTR* 2756A>G, *MTRR* 66A>G, *CBS* 844ins68, *TYMS* 1494del6, and *DHFR* c.86+60_78). All genotypes were in Hardy-Weinberg equilibrium for SLE patients and controls in the total study population and after stratification by race (data not shown). The genotype frequency distributions were significantly different between African American and Caucasian controls for all of the polymorphisms except for *MTR* 2756A>G by chi-square test (Table 3).

SLE and polymorphisms of folate/homocysteine-metabolizing enzymes. Neither the genotype frequency distributions of the 7 polymorphisms (data not shown) nor the carrier frequency distributions of each individual polymorphism stratified by race (Table 4) differed between SLE patients and controls by chi-square or Fisher's exact tests.

Predictors of homocysteine levels. The continuous variables

Table 2. Sample characteristics.

Characteristic	SLE Patients	Controls	p
No.	163	160	ND
Age, yrs, mean ± SD	43.3 ± 11.0	43.5 ± 10.5	0.90
Race, % (n)			
African American	50.3 (82)	50.6 (81)	1.00
Caucasian	39.3 (64)	39.4 (63)	
Asian	4.9 (8)	5.0 (8)	
Hispanic	4.3 (7)	3.8 (6)	
Other	1.2 (2)	1.2 (2)	
CAC, median (IQR)	0 (0–7.2)	0 (0–0)	0.0003
Homocysteine, µmol/l, mean ± SD (n)	10.4 ± 1.4	9.2 ± 1.4	< 0.0001
African American*	12.1 ± 1.4 (82)	9.7 ± 1.3 (81)	< 0.0001
Caucasian	10.0 ± 1.3 (64)	9.0 ± 1.5 (63)	0.12
Framingham point scores, median (IQR) (n)	8 (3–13) (161)	8 (2–12) (156)	0.39
GFR, ml/min/1.73m^2, mean ± SD	90.0 ± 32.5	94.5 ± 20.8	0.14

CAC: coronary artery calcification; GFR: glomerular filtration rate. ND: not determined; IQR: interquartile range. * African American case vs Caucasian case, p = 0.0009; African American control vs Caucasian control, p = 0.21.

Table 3. Distribution of genotype frequencies in African American and Caucasian controls.

Polymorphism	Genotype	African American	Caucasians	p
MTHFR 677C>T	CC	67.9 (55)	39.7 (25)	0.0003
	CT	29.6 (24)	41.3 (26)	
	TT	2.5 (2)	19.0 (12)	
MTHFR 1298A>C	AA	69.1 (56)	55.6 (35)	0.0347
	AC	30.9 (25)	38.1 (24)	
	CC	0	6.3 (4)	
MTHFR 677/1298	CC/AA	46.9 (38)	11.1 (7)	< 0.0001
	CC/AC	21.0 (17)	22.2 (14)	
	CC/CC	0	6.3 (4)	
	CT/AA	19.7 (16)	25.4 (16)	
	CT/AC	9.9 (8)	15.9 (10)	
	TT/AA	2.5 (2)	19.0 (12)	
CBS 844ins68	WW	58.0 (47)	87.3 (55)	0.0005
	WI	39.5 (32)	12.7 (8)	
	II	2.5 (2)	0	
MTR 2756A>G	AA	58.0 (47)	47.6 (30)	0.19
	AG	33.3 (27)	47.6 (30)	
	GG	8.7 (7)	4.8 (3)	
MTRR 66A>G	AA	48.2 (39)	20.6 (13)	0.0009
	AG	40.7 (33)	50.8 (32)	
	GG	11.1 (9)	28.6 (18)	
TYMS 1494del6	Ins/ins	19.7 (16)	39.7 (25)	0.0012
	Ins/del	53.1 (43)	54.0 (34)	
	Del/del	27.2 (22)	6.3 (4)	
DHFR c.86+60_78	Ins/ins	19.7 (16)	38.1 (24)	0.0068
	Ins/del	45.7 (37)	47.6 (30)	
	Del/del	34.6 (28)	14.3 (9)	

Genotype frequencies % (n). P values by chi-square test.

Table 4. Distributions of carrier frequencies between SLE patients and controls.

Genotype	SLE Patients	Controls	p
MTHFR 677T carriers			
African American	31.7 (26)	32.1 (26)	1.00
Caucasian	65.6 (42)	60.3 (38)	0.58
MTHFR 1298C carriers			
African American	32.9 (27)	30.9 (25)	0.87
Caucasian	54.7 (35)	44.4 (28)	0.29
CBS 844ins68 carriers			
African American	48.8 (40)	42.0 (34)	0.43
Caucasian	15.6 (10)	12.7 (8)	0.80
MTR 2756G carriers			
African American	46.3 (38)	42.0 (34)	0.64
Caucasian	35.9 (23)	52.4 (33)	0.07
MTRR 66G carriers			
African American	43.9 (36)	51.9 (42)	0.35
Caucasian	78.1 (50)	79.4 (50)	1.00
TYMS 1494del6 ins carriers			
African American	63.4 (52)	72.8 (59)	0.24
Caucasian	89.1 (57)	93.7 (59)	0.53
DHFR c.86+60_78 ins carriers			
African American	59.8 (49)	65.4 (53)	0.52
Caucasian	78.1 (50)	85.7 (54)	0.36

Carriers of alleles subset by race % (n). P values by Fisher's exact test.

selected for correlation analysis with homocysteine were age, GFR, and Framingham point scores. Pearson coefficients and p values are given in Table 5; all 3 continuous variables were significantly correlated with homocysteine. Age and Framingham point scores were positively correlated while GFR was negatively correlated with homocysteine levels. Categorical variables selected for analysis by Student t-test for independent samples were folic acid use, B_6 use, B_{12} use, and smoking status, which were uncontrolled sources of variation. None of the variables were significantly associated with homocysteine ($p > 0.05$). The results were similar when stratified by group (SLE patients vs controls) except for smoking status, which was significantly associat-

Table 5. Correlations with homocysteine levels.

Variable	Pearson Correlation Coefficient	n	p
Age	0.22917	323	< 0.0001
GFR	–0.42930	323	< 0.0001
Framingham point scores	0.22249	317	< 0.0001

GFR: glomerular filtration rate.

ed with mean homocysteine only in controls [smokers 11.8 ± 1.3 (n = 25) vs nonsmokers 9.5 ± 1.4 (n = 133); p = 0.0046].

As discussed above there were differences in homocysteine levels by group and by race, which consisted of African Americans and Caucasians. From the above analysis the explanatory variables age, GFR, and Framingham point scores along with race and group were put into a general linear model with homocysteine as the dependent variable, with no interactions between any of the terms. GFR along with group and race were the only variables significantly associated with homocysteine (Table 6). These variables were used in the modeling of the effect of genotype on homocysteine.

Modeling the effects of polymorphisms of folate/homocysteine-metabolizing enzymes on homocysteine concentrations. After exploring more complex models we arrived at a clinically sound parsimonious model with variables that significantly contributed to the variation in homocysteine. A general linear model with homocysteine as the dependent variable and classified by race, group, and genotype and all possible interactions with the addition of the covariate GFR and its interaction with genotype was used for each polymorphism based on the above analysis, which determined these variables to be significantly associated with homocysteine concentrations. The 4 categories analyzed were African American SLE patients, African American controls, Caucasian SLE patients, and Caucasian controls. The least-square mean estimates of homocysteine were adjusted for the uncontrolled variable GFR. Two of the polymorphisms (*MTHFR* 1298 and *CBS* 844ins68) were modeled based on the combination of the heterozygotes with the homozygotes because the homozygotes for the polymorphism were not present in at least one of the 4 categories. Out of the 7 polymorphisms only 2 had significant results, both in Caucasian controls (Table 7). For *MTHFR* 677 CC vs CT and CC vs TT the homocysteine concentrations were 7.7 versus 9.4 (p = 0.0196) and 7.7 versus 9.8 μmol/l (p = 0.0275), respectively. For *MTHFR* 1298 AA versus AC/CC the homocysteine concentrations were 9.6 versus 7.8 μmol/l (p = 0.0083), respectively.

CAC scores and polymorphisms of folate/homocysteine-metabolizing enzymes. None of the genotypes under test was associated with median CAC scores by Kruskal-Wallis test even after stratification by race (data not shown).

Table 6. Analysis of covariance for homocysteine.

Source Variation	Sum of Squares	Df	F	p
GFR	1.11	1	60.18	< 0.0001
Group*	5.53	1	12.04	0.0006
Race†	2.14	1	23.28	< 0.0001

* Case and control groups. † African Americans and Caucasians.

Table 7. Analysis of covariance with 2 factors (group, race) and one covariate (glomerular filtration rate).

Category	Genotype	Adjusted Means of Homocysteine	p*
Caucasian controls	MTHFR 677 CC	7.7	—
	CT	9.4	0.0196
	TT	9.8	0.0275
Caucasian controls	MTHFR 1298 AA	9.6	—
	AC/CC†	7.8	0.0083

* p value for comparison to wild-type genotype. † These genotypes were combined because of low numbers of homozygotes.

DISCUSSION

Our a priori hypothesis was that established functional polymorphisms of enzymes in the folate/homocysteine pathway would be associated with increased homocysteine concentrations in patients with SLE, and hence with CAC scores, a clinical finding for which elevated homocysteine concentrations are predictive[19]. The polymorphisms selected for this study (*MTHFR* 677C>T and 1298A>C, *MTR* 2756A>G, *MTRR* 66A>G, *CBS* 844ins68, *TYMS* 1494del6, and *DHFR* c.86+60_78) were those for which there is evidence for significant effects on homocysteine concentrations[7-16]. The differences in the distributions of genotype frequencies between African American and Caucasian controls mandated that associations of homocysteine with genotype be stratified by race. More specifically, it is known that *MTHFR* 677C>T and 1298A>C[28], *MTRR* 66A>G[28], CBS 844ins68[29,30], and *TYMS* 1494del6[30] have different genotype frequencies in African Americans and Caucasians. Our study also found that frequencies of *DHFR* c.86+60_78 differed between races.

None of the genotypes for the polymorphisms under test differed significantly in distribution between SLE patients and controls, even after stratification by race, indicating that none are genetic risk factors for SLE per se. A study by Fijnheer, *et al*[31] found that *MTHFR* 677C>T did not explain elevated homocysteine levels in SLE patients, which agrees with our findings. Our results contrast with a smaller Italian study reporting that SLE patients had a higher prevalence of the *MTHFR* 677TT genotype[18]. A Polish study found that frequencies of *MTHFR* 677C>T were not different between SLE patients and controls, but the authors found that the *MTR* 2756G allele was overrepresented in SLE patients[32], which contrasts with our finding of lack of an association.

Although folate, B_{12}, and B_6 levels were not available for our study, use of folic acid, B_{12}, and B_6 supplements was studied as a surrogate, but was found to be not associated with homocysteine concentrations. GFR was correlated negatively with homocysteine, and other studies have found a similar relationship[33]. In SLE patients none of the 7 polymorphisms were associated with homocysteine levels. In controls there were 2 polymorphisms associated with homo-

cysteine levels, but only in Caucasians. The *MTHFR* 677CT and TT genotypes were associated with an increase in homocysteine levels compared to CC, as expected from the literature[7,8]. Carriers of the *MTHFR* 1298C allele had lower homocysteine levels compared to AA. This finding is concordant with a study by Parle-McDermott, *et al* that found that *MTHFR* 1298AC and CC genotypes were associated with increased red-cell folate and a nonsignificant decrease in homocysteine within the *MTHFR* 677CC genotype in pregnant women[34]. A large study by Ulvik, *et al* used an approach similar to the Parle-McDermott study and stratified their analysis of biochemical variables based on both *MTHFR* 677 and 1298 genotypes. Ulvik, *et al* found that *MTHFR* 1298AC and CC genotypes were associated with higher homocysteine levels and lower serum folate levels[9]. The differences in findings among the studies on *MTHFR* 1298A>C may be due to the number of subjects studied or to the genetic variability of the populations studied.

Although our study has some limitations, the magnitude of the difference in homocysteine concentrations between SLE patients and controls was large enough to suggest that genetic factors might be responsible, at least in part, for the elevation of homocysteine in the patients. Such a genetic effect (i.e., the *MTHFR* 677C>T polymorphism) was observed in the controls, but not in patients with SLE. While we acknowledge that the size of our study population precludes a conclusion that there are no contributing genetic factors, our study suggests that if such factors are involved they are likely to have no more than a relatively small effect. Thus, the increase in homocysteine among SLE patients is probably due primarily to other variables that are components of the SLE disease process itself.

African American patients with SLE had elevated homocysteine levels compared to Caucasian patients and African American controls. None of the tested functional polymorphisms of enzymes in the folate/homocysteine pathway were associated with SLE. In addition, in SLE patients, none of the polymorphisms were associated with homocysteine concentrations even when adjusted for covariates, including GFR, and there were no associations with median CAC scores. It is unlikely that polymorphisms in folate/homocysteine-metabolizing enzymes contribute substantially to the elevated homocysteine levels observed in patients with SLE. Therefore the mechanism whereby SLE patients achieve elevated homocysteine concentrations and high CAC scores relative to controls is most likely due to inflammatory aspects of the disease process that dominate any genetic effects intrinsic to enzymes of the folate/homocysteine pathway.

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