Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2008 October ; 82(10): 736–741. doi:10.1002/bdra.20507.

Genetic and Biochemical Determinants of Serum Concentrations of Monocyte Chemoattractant Protein-1, a Potential Neural Tube Defect Risk Factor

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Abstract

Background—Women with the AA genotype at the (–2518) A>G promoter polymorphism of *CCL-2*, which encodes the potent pro-inflammatory chemokine monocyte chemoattractant protein 1 (MCP-1), may be at increased risk for having offspring affected by spina bifida. As the A allele at this locus has been associated with decreased transcription of MCP-1 mRNA relative to the G allele, the observed genetic association suggests that the risk of spina bifida may be increased in the offspring of women with low MCP-1 levels. The present study was undertaken to identify potential determinants of MCP-1 levels in women of reproductive age.

Methods—A small cohort of Caucasian and African-American women of reproductive age was recruited to participate in an exploratory investigation of the determinants of several disease-related, biochemical phenotypes, including MCP-1. Subjects completed a brief questionnaire and provided a fasting blood sample for biochemical and genetic studies. Potential biochemical, genetic and lifestyle factors were assessed for their association with MCP-1 levels using linear regression analyses.

Results—In this cohort, MCP-1 levels were significantly higher in Caucasians as compared to African-Americans. Further, among women of both races, there was evidence that MCP-1 levels were associated with smoking status, *MTHFR* 677C>T genotype and red blood cell tetrahydrofolate levels.

Conclusions—The results of these analyses indicate that, if maternal *CCL-2* genotype is related to the risk of spina bifida, this relationship is likely to be more complex than initially hypothesized, perhaps depending upon folate intake, *MTHFR* 677C>T genotype, the distribution of folate derivatives, and immune/inflammatory activity.

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The data in this manuscript have not been presented in full or in part at any meeting

Keywords

CCL-2; monocyte chemoattractant protein-1; folate; genetics; inflammation; neural tube defects; spina bifida

INTRODUCTION

In humans, defects of neurulation are relatively common and result in serious malformations, including anencephaly and spina bifida (Mitchell and others, 2004). Collectively, these malformations are referred to as neural tube defects (NTDs). While a small proportion of fetuses/infants affected by an NTD are identified as having an underlying syndrome, no specific cause(s) can be identified in the majority. A portion of NTDs in this latter group can be prevented by maternal periconceptional supplementation with folic acid (Czeizel and Dudas, 1992; MRC Vitamin Study Research Group, 1991), and in the absence of such supplementation, pregnancies that result in an NTD birth outcome are characterized by low maternal folate status (Kirke and others, 1993; Mills and others, 1995). This suggests that folic acid is corrective rather than pharmacologically active, and therefore that there may be a causative relationship between maternal folate insufficiency (or dysregulation) and failure of neurulation. However, the precise mechanism(s) by which low folate status contributes to NTD etiology remains unknown.

The protective effect of maternal periconceptional folic acid has generated considerable interest in the identification of genetic variants that are associated with the risk of NTDs due to their impact on folate transport, metabolism or excretion (Beaudin and Stover, 2007). However, only one such variant, the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) 677C>T single nucleotide polymorphism (SNP), has been strongly (although not unequivocally) implicated as an NTD risk factor (Barber and others, 2000; Shields and others, 1999). Consequently, there is interest in expanding the list of genetic candidates for NTDs to include genes that are biologically linked to other known or suspected NTD risk factors, including maternal obesity, diabetes, and hyperthermia.

Many of the known NTD risk factors (e.g. diabetes) have inflammatory features, suggesting that genes involved in the inflammatory response may be involved in the etiology of this group of conditions. Interestingly, it has been shown, in a cultured endothelial cell line, that folate insufficiency induces increased synthesis and export of monocyte chemoattractant protein 1 (MCP-1), a potent pro-inflammatory chemokine (Brown and others, 2006). In turn, MCP-1 acts in an autocrine fashion to elicit changes in cell morphology, including the acquisition of actin stress fibers (Brown and others, 2006). Since actin dynamics underpin cellular shape changes such as those required for convergent extension during neurulation (Schoenwolf and Smith, 1990), alterations of MCP-1 levels could have a direct effect on morphogenesis. Furthermore, MCP-1, together with other chemokines and cytokines, appears to be important in signaling between the embryo and endometrium during implantation and placentation (Kayisli and others, 2002), which occurs just prior to neurulation. Hence, altered MCP-1 expression could influence the risk of NTDs through modulation of maternal inflammatory responses.

The (−2518) A>G promoter polymorphism of *CCL-2*, the gene encoding MCP-1, confers differential responsiveness to Interleukin-1 (IL-1) (Rovin and others, 1999) and is, therefore, a logical NTD genetic candidate that is only indirectly related to folate metabolism. Evaluation of this polymorphism in a large number of spina bifida case-parent triads indicated that maternal *CCL-2* genotype is associated with the risk of spina bifida in offspring (Jensen and others, 2006). Specifically, women with the *CCL-2* (−2518) AA

genotype appear to be at higher risk of having offspring affected with spina bifida than women with the AG or GG genotypes. As monocytes from *CCL-2* AA homozygotes are known to produce less MCP-1 (as compared to those from *CCL-2* AG heterozygotes or GG homozygotes) in response to IL-1, the observed increased risk of spina bifida in the offspring of women with the *CCL-2* (-2518) AA genotype was hypothesized to be due to a sub-optimal systemic and/or local immune or inflammatory response resulting from low MCP-1 levels at the time of neural tube closure. However, as the *CCL-2* (-2518)A>G polymorphism is not the only determinant of MCP-1 levels this explanation may be overly simplistic.

In addition to the *CCL-2* (-2518)A>G polymorphism, variables that have been associated with MCP-1 levels include sex, age, race, diabetes, obesity, smoking status and the region of chromosome 3 that contains the chemokine receptor gene cluster, which includes the receptor for MCP-1 (Bielinski and others, 2007; McDermott and others, 2005). However, to our knowledge, there are no published studies that have focused on the potential determinants of MCP-1 levels in reproductive age females. The present analyses were, therefore, undertaken to explore genetic and environmental variables that might influence MCP-1 levels in women at risk of having an NTD affected pregnancy.

MATERIALS AND METHODS

Study Subjects

Pre-menopausal female subjects were recruited by advertisement from staff and students at the University of Pennsylvania School of Medicine, from January 9, 2007 to July 26, 2007. Potential study subjects were excluded if they had a major medical condition (e.g. autoimmune disease), were using an anti-folate medication or disease modifying anti-rheumatoid drugs, or were pregnant. The study was approved by the Institutional Review Board of the University of Pennsylvania School of Medicine, and all subjects provided informed consent.

Study subjects attended two study visits. The analyses presented here are based on values obtained at the first visit, during which subjects provided a fasting blood sample in the morning and completed a short, in-person interview that included questions related to use of alcohol, smoking status, height and weight.

Laboratory Methods

Serum MCP-1 levels were measured using a human MCP-1 ELISA kit (BD Biosciences) according to the manufacturer's instructions.

Total homocysteine (tHcy) and both plasma and red blood cell (RBC) folate derivatives were measured using stable isotope dilution liquid chromatography, multiple reaction monitoring, mass spectrometry (LC/MRM/MS) as previously described (Huang and others, 2008; Huang and others, 2007). The measured folate derivatives were 5-methyltetrahydrofolate (5-MTHF), tetrahydrofolate (THF) and 5,10-methenyltetrahydrofolate (5,10-MTHF).

Levels of C-reactive protein were measured in the clinical laboratory of the Hospital of the University of Pennsylvania using VITROS MicroSlides (Ortho-Clinical Diagnostics).

Genotyping

DNA was extracted from whole blood using the QIAamp® DNA Mini Kit (Qiagen). *MTHFR* 677C>T, *MTHFR* 1298 A>C and *CCL-2* (-2518) A>G allelic discrimination was

performed using TaqMan 5' Nuclease Real-Time PCR assays on a DNA Engine Opticon 2 Continuous Fluorescence Detection System (MJ Research, Waltham, MA). Probes were custom synthesized by Applied Biosystems. In each case, dual fluorescence was detected after each extension 5' nuclease step, and genotype interpretations were performed using OpticonMonitor Analysis software version 2.02 (MJ Research).

For *MTHFR* 677C>T genotyping, PCR amplifications were performed as described previously (Huang and others, 2008). Briefly, 4-25ng of sample DNA, 0.5μM each of forward (5'-GCAGGGAGCTTTGAGGCTGACC-3') and reverse (5'-TGGGGCAAGTGATGCCCATGT-3') primers, together with 50nM "T"-allele probe (5'-6FAM-ATGAAATCGACTCCCGC-3'-MGBNFQ) and 100nM "C"-allele probe (5'-VICATGAAATCGGCTCCCGC-3'-MGBNFQ) were combined in 20μl 1× Taqman Universal PCR MasterMix (Applied Biosystems, Foster City, CA). PCR was performed with an initial incubation at 95°C for 10 min, followed by 60 cycles of denaturation at 95°C for 30 sec and extension/5' nuclease step at 56°C for 1 min.

For *MTHFR* 1298A>C genotyping, PCR amplifications were performed as described previously (Summers and others, 2008). Briefly, 4-25ng of sample DNA, 0.5μM each of forward (5'-GAGGAGCTGCTGAAGATGT-3') and reverse (5'-CGAGAGGTAAAGAACGAAGA-3') primers, together with 50nM each of "T"-allele probe (5'-6FAM-AGACACTTGCTTCACT-3'-MGBNFQ) and "C"-allele probe (5'-VICCAAAGACACTTTCTTC-3'-MGBNFQ) were combined in 20μl 1× Taqman Universal PCR MasterMix (Applied Biosystems). PCR was performed with an initial incubation at 92°C for 10 min, followed by 60 cycles of denaturation at 92°C for 1 min and extension/5' nuclease step at 60°C for 1 min.

For *CCL-2* (-2518) A>G genotyping, PCR amplifications were performed as described previously (Jensen and others, 2006) with minor modification. Briefly, 4-25ng genomic DNA, 0.5μM each of forward (5'-TTCTTGACAGAGCAGAAGTGG-3') and reverse (5'-GCCTTTGCATATATCAGACAGTA-3') primers, together with 50nM each of "A"-allele probe (5'-6FAM-AGACAGCTATCACTT-3'-MGBNFQ) and "G"-allele probe (5'-VIC-AGACAGCTGTCACTTTC-3'-MGBNFQ) were combined in 20μl Taqman master mix (Applied Biosystems). PCR was performed with an initial incubation at 95°C for 10 min, followed by 60 cycles of denaturation at 95°C for 15 seconds and extension/5' nuclease step at 57°C for 30 seconds.

Statistical Methods

Descriptive analyses of the study variables included counts and proportions for discrete variables, and means and standard deviations for continuous variables. Body mass index (BMI) was calculated as: $\text{weight(kg)}/[\text{height(m)}]^2$, and total RBC folate as the sum of RBC 5-MTHF, THF and 5,10-MTHF. Given the small study sample size and consequent low power to detect departures from normality, the distribution of MCP-1 levels was assessed by visual inspection of the data. Simple linear regression analyses were performed with MCP-1 levels as the outcome measure. The coefficient of determination (R^2) estimated from these models was used to assess the proportion of variation in MCP-1 levels that was explained by each predictor variable. The significance of the association between each predictor variable and MCP-1 levels was assessed using the t-statistic. All analyses were performed separately by race, using data obtained during the first study visit and SAS version 9.1 (SAS Institute, Inc, Cary, NC). Values of $R^2 > 0.10$ and p-values < 0.10 were considered of interest. However, due to the relatively small sample size and the number of comparisons that were evaluated, these analyses should be considered exploratory in nature and the significance of any single test result must be considered in this context.

RESULTS

A total of 53 women consented to participate in this study. However, four (7%) were subsequently found to have medical conditions and/or to be taking medications that fell within the study exclusion criteria. The data from these four women were excluded from all analyses. Among the remaining 49 women, mean age was 32.5 years (range: 22.2-49.1 years) at the first study visit, and self-reported race was Caucasian in 26 (53%) and African American in 23 (47%). None of the study subjects reporting having a pregnancy affected by spina bifida.

The characteristics of the study subjects at the time of the first study visit are summarized, separately by race, in Table 1. Upon visual inspection, the distribution of MCP-1 values did not deviate markedly from normality. It is, however, of note that the distribution of MCP-1 in Caucasians more closely approximated a normal distribution than that in African-Americans. As MCP-1 levels were significantly lower in African American as compared to Caucasian subjects ($t=2.46$, $p=0.02$ from simple linear regression of race on MCP-1), and several of the potential predictor variables were also distributed quite differently in African Americans and Caucasians (Table 1), all analyses were performed separately by race.

Subject Characteristics

MCP-1 concentrations were not associated with age BMI, parity or use of multivitamin, B complex or folic acid supplements in either African-American or Caucasian study subjects (Table 2). However, MCP-1 levels were associated with current smoking status ($R^2=0.13$); MCP-1 concentrations were higher among smokers in both races (Tables 2 and 3). In Caucasians, but not African-Americans, alcohol use also was associated with MCP-1 levels. However, this association may reflect an inverse association between smoking status and alcohol use (i.e. smokers reported less alcohol use than non-smokers in both racial groups) that was stronger in the Caucasian (odd ratio (OR)=0.03, 95% confidence interval (CI) 0.002-0.49) than in the African-American (OR=0.36, 95% CI 0.04-3.26) subjects.

Biochemical Phenotypes

MCP-1 concentrations were not associated with tHcy, RBC folate, RBC 5-MTHF, RBC 5,10-MTHF, plasma 5-MTHF or C-reactive protein (Table 2). Among African-American women, MCP-1 levels were inversely associated with RBC THF ($R^2=0.15$) and the ratio of RBC THF to RBC 5-MTHF ($R^2=0.22$). In Caucasian women, MCP-1 levels were associated with RBC THF ($R^2=0.11$) and the ratio of RBC THF to RBC 5-MTHF ($R^2=0.13$), and the direction of these associations was the opposite of that observed in the African-American subjects.

Genotypes

MCP-1 levels were not associated with *CCL-2* (-2518) A>G genotypes, but were associated with *MTHFR* 677C>T genotypes in both African-American and Caucasian subjects (Tables 2 and 3). In both races, the *MTHFR* 677T allele was associated with increased MCP-1 levels. In African-Americans this allele appeared to have a dominant or co-dominant effect on MCP-1 levels (i.e. MCP-1 levels were increased in women with the CT as compared to CC genotype; there were no African-American women with the TT genotype in the study population). In Caucasians, the effect of the *MTHFR* 677T allele on MCP-1 levels appeared to be recessive (i.e. compared to women with the CC genotype, MCP-1 levels in women with the TT, but not the CT genotype, were increased). Although the effect of the *MTHFR* 677T allele appears to differ by race, it is possible that there is a dose response relationship between this allele and MCP-1 levels in both races, but that this relationship is obscured by

relatively small numbers in each genotype category and the absence of the *MTHFR* 677TT genotype in the African-American subjects.

Among Caucasians, the *MTHFR* 1298A>C genotype was also associated with MCP-1 levels. However, since a similar association was not observed in African-Americans, this may be attributable to linkage disequilibrium between the two *MTHFR* variants.

DISCUSSION

In this small study of premenopausal women, MCP-1 levels were significantly lower in African-Americans as compared to Caucasians. Lower levels of MCP-1 in African-Americans relative to Caucasians have been previously reported (Bielinski and others, 2007). In both races, the strongest predictors of MCP-1 levels appeared to be current smoking status, *MTHFR* 677C>T genotype and RBC THF levels. An association between MCP-1 levels and smoking status has also been previously reported (Bielinski and others, 2007; McDermott and others, 2005). We are unaware of previous reports indicating that MCP-1 levels are associated with either *MTHFR* genotype or RBC THF levels. However, in an experimental model of chronic mild folate depletion in endothelial cells, MCP-1 mRNA and protein synthesis were up-regulated (Brown and others, 2006). Hence, the observed association of MCP-1 levels with *MTHFR* 677C>T and RBC THF, provides further support for the hypothesis that perturbations in folate/homocysteine metabolism contribute to the induction of MCP-1 expression (Brown and others, 2007).

Given the relatively small samples sizes available in this study, it was not possible to evaluate whether the observed association between MCP-1 levels and *MTHFR* 677C>T genotype or between MCP-1 levels and RBC THF levels were independent of each other. In addition, it was not possible to investigate the source of the difference in the observed association between RBC THF and MCP-1 levels in the African-American and Caucasian subjects. This difference may, however, be related to differences in the *MTHFR* 677C>T genotype distribution between Caucasians and African-Americans.

There was no evidence that MCP-1 levels in our subjects were associated with the *CCL-2* (–2518) A>G polymorphism. The lack of such an association is not entirely surprising, given the presumptive absence of overt inflammatory stimuli in these study subjects. Indeed there is evidence from cell culture studies that pro-inflammatory stimuli, such as TNF- α , induce *CCL-2* transcription via NF- κ B, whereas folate insufficiency (under non-inflammatory conditions) induces *CCL-2* transcription via a p38 dependent mechanism (Lu and others, unpublished). The two pathways appear to be distinct, but to have multiplicative effects when both are engaged (Lu and others, unpublished). The *CCL-2* (–2518) G allele confers a cytokine dependent transcriptional advantage (Rovin and others, 1999) and hence this variant would be expected to be associated with elevated MCP-1 levels *in vivo* only in the presence of inflammation. It is therefore likely that MCP-1 levels in our healthy subjects are being determined by the folate-dependent p38-mediated pathway, which does not appear to be influenced by the *CCL-2* promoter polymorphism.

Several additional variables that have been reported to be significantly related to MCP-1 levels (e.g. age, BMI) were not identified as significant predictors of MCP-1 levels in this cohort. Given the small sample sizes in the present study, it is possible that some associations have been missed due to low study power. However, it is also possible that differences in findings between these and other studies reflect differences in the characteristics across study populations. The present study was based on healthy, reproductive age females, whereas many of the other published studies of the determinants of MCP-1 levels have focused on cohorts with a specific disease phenotype (e.g.

cardiovascular disease, systemic lupus erythematosus), and included a broader age range, an older cohort, and/or both sexes (Bielinski and others, 2007; Brown and others, 2007; McDermott and others, 2005).

We have previously reported that women with the *CCL-2* (−2518)AA genotype are at increased risk for having offspring affected with spina bifida. As the *CCL-2* A allele has been associated with decreased transcription and lower circulating levels of MCP-1 under inflammatory conditions, we speculated that low MCP-1 levels might also be associated with the risk of spina bifida, possibly due to a less than optimal systemic and/or local response to infection early in the first trimester of pregnancy (Jensen and others, 2006). While this study does not directly address the above hypothesis, we have investigated the relationship between several established (i.e. race, folate status) or strongly suspected (i.e. *MTHFR* 677C>T genotype) NTD risk factors and MCP-1 levels. Interestingly, MCP-1 levels were *higher* in the subgroup of women who are at higher risk of having NTD affected offspring based on race (i.e. Caucasians) and *MTHFR* 677C>T genotype (i.e. *MTHFR* 677TT), a finding that appears contrary to expectations based on the above hypothesis. If the maternal *CCL-2* (−2518)A>G genotype is related to the risk of spina bifida, this finding could indicate that any effect of MCP-1 levels on the risk of NTDs, is independent of these other risk factors, or that this relationship may be more complex than suggested by our initial hypothesis, perhaps depending upon folate intake, genetically mediated distribution of folate derivatives, and immune/inflammatory activity. Such complexity seems likely given the separate folate-dependent and inflammation-dependent mechanisms that control MCP-1 expression.

Acknowledgments

This work was supported by grants AR47663, P20RR020741, HD039195, and ES013508 from the National Institutes of Health, and by grant 4100038714 from the Pennsylvania Department of Health

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Table 1

Subject characteristics, biochemical phenotypes and genotypes (mean±s.d. or count and percentage).

Variable	Race	
	African-Americans (N = 23)	Caucasians (N = 26)
Subject Characteristics		
Age (years)	31.6 ± 6.0	33.3 ± 6.5
Body mass index (kg/m ²)	28.3 ± 5.9	23.5 ± 3.4
Body mass index		
18.5-24.9 kg/m ² (normal)	6 (26.1)	21 (80.8)
25.0-29.9 kg/m ² (overweight)	11 (47.8)	4 (15.4)
>30 kg/m ² (obese)	6 (26.1)	1 (3.8)
Parity		
0	8 (34.8)	17 (65.4)
≥1	15 (65.2)	9 (34.6)
Vitamins ^I		
Yes	8 (34.8)	9 (34.6)
No	15 (65.2)	17 (65.4)
Cigarettes		
Yes	4 (17.4)	5 (19.2)
No	19 (82.7)	21 (80.8)
Alcohol intake		
Yes	16 (69.6)	22 (84.6)
No	7 (30.4)	4 (15.4)
Biochemical Phenotypes		
MCP-1 (pg/mL)	164.4 ± 110.3	244.4 ± 116.3
Homocysteine (μmol/L)	8.9 ± 2.5	9.6 ± 2.7
RBC folate (nmol/L) ²	937.5 ± 341.0	1185.5 ± 329.1
RBC 5-MTHF (nmol/L)	919.3 ± 334.1	1040.3 ± 333.0
RBC THF (nmol/L)	17.5 ± 11.1	117.8 ± 214.5
RBC 5,10-MTHF		
Not detected	21 (91.3)	10 (38.5)
>0 nmol/L	2 (8.7)	16 (61.5)
RBC THF:5-MTHF	0.02 ± 0.01	0.2 ± 0.5
Plasma 5-MTHF (nmol/L)	33.5 ± 17.2	48.4 ± 20.5
C-reactive protein		
≤0.9 mg/dL	4 (17.4)	2 (7.7)
>0.9 mg/dL	19 (82.6)	24 (92.3)
Genotypes		
<i>CCL-2</i> (-2158)A>G		
AA	16 (69.6)	13 (50.0)
AG	7 (30.4)	10 (38.5)
GG	0 (0.0)	3 (11.5)

Variable	Race	
	African-Americans (N = 23)	Caucasians (N = 26)
<i>MTHFR</i> 677C>T		
CC	16 (69.6)	8 (30.8)
CT	7 (30.4)	13 (50.0)
TT	0 (0.0)	5 (19.2)
<i>MTHFR</i> 1298A>C		
AA	13 (56.5)	14 (53.8)
AC	10 (43.5)	10 (38.5)
CC	0 (0.0)	2 (7.7)

¹ Vitamins include: multivitamin, B complex or folic acid supplements

² RBC folate = (RBC 5-MTHF) + (RBC THF) + (RBC 5,10-MTHF)

Table 2

Proportion of variation (R^2) in MCP-1 levels explained by selected subject characteristics, biochemical phenotypes and genotypes, by race.

Variables	Race			
	African-American (N=23)		Caucasian (N=26)	
	Variable Coefficient (SE)	R ² (P-value)	Variable Coefficient (SE)	R ² (P-value)
Subject Characteristics				
Age (years)	-1.0 (4.0)	0.003 (0.80)	2.7 (3.6)	0.02 (0.46)
Body mass index (kg/m ²)	3.7 (4.0)	0.04 (0.36)	-5.3 (6.8)	0.02 (0.45)
Parity (0 versus ≥1)	73.4 (46.8)	0.10 (0.13)	28.3 (48.6)	0.01 (0.56)
Vitamins (yes/no) ¹	-0.16 (49.4)	0.00 (1.00)	60.3 (47.4)	0.06 (0.22)
Cigarettes (yes/no)	102.6 (58.0)	0.13 (0.09)	103.2 (55.2)	0.13 (0.07)
Alcohol (yes/no)	-31.8 (50.7)	0.02 (0.54)	-118.7 (59.8)	0.14 (0.06)
Biochemical Phenotypes				
Homocysteine (μmol/L)	-3.4 (9.7)	0.01 (0.73)	4.2 (8.9)	0.01 (0.64)
RBC folate (nmol/L) ²	-0.06 (0.07)	0.04 (0.38)	0.04 (0.07)	0.02 (0.54)
RBC 5-MTHF (nmol/L)	-0.06 (0.07)	0.03 (0.41)	-0.05 (0.07)	0.02 (0.52)
RBC THF (nmol/L)	-3.8 (2.0)	0.15 (0.07)	0.2 (0.1)	0.11 (0.10)
RBC 5,10-MTHF ³	-61.7 (82.5)	0.03 (0.46)	8.0 (47.8)	0.00 (0.87)
RBC THF : RBC 5-MTHF	-4637.7 (1914.2)	0.22 (0.02)	86.3 (44.7)	0.13 (0.07)
Plasma 5-MTHF (nmol/L)	-1.3 (1.4)	0.04 (0.35)	-0.9 (1.1)	0.03 (0.44)
C reactive protein (mg/dL) ⁴	-37.0 (61.6)	0.02 (0.55)	118.6 (83.9)	0.08 (0.17)
Genotypes				
<i>CCL-2</i> (-2518)A>G ⁵				
AG	10.1 (51.1)	0.002 (0.85)	-28.8 (50.4)	0.02 (0.77)
GG	-		19.8 (76.8)	
<i>MTHFR</i> 677C>T ⁵				
CT	76.1 (48.4)	0.11 (0.13)	-7.8 (49.1)	0.19 (0.09)
TT	-		119.8 (62.3)	
<i>MTHFR</i> 1298A>C ⁵				
AC	-29.5 (47.1)	0.02 (0.54)	-91.6 (46.0)	0.16 (0.13)
CC	-		-92.1 (84.0)	

¹ Vitamins include: multivitamin, B complex or folic acid supplements

² RBC folate = (RBC 5-MTHF) + (RBC THF) + (RBC 5,10-MTHF)

³ Not detected versus >0 nmol/L

⁴ <9mg/dL versus >9mg/dL

⁵ The homozygous *MTHFR* 677TT genotype was not observed among African-American subjects

Table 3

Mean MCP-1 level by race and other covariates

Variables	Mean MCP-1 Level, pg/mL (N)	
	African-Americans	Caucasians
Total	164.4 (23)	244.4 (26)
Cigarettes		
Yes	249.2 (4)	327.7 (5)
No	146.6 (19)	224.6 (21)
RBC THF ^I		
≤50 th percentile	175.6 (12)	215.3 (13)
>50 th percentile	152.2 (11)	273.5 (13)
RBC 5-MTHF ^I		
≤50 th percentile	172.6 (12)	249.0 (13)
>50 th percentile	155.5 (11)	239.8 (13)
<i>MTHFR</i> 677C>T		
CC	141.3 (16)	225.3 (8)
CT	217.3 (7)	217.4 (13)
TT	-	345.1 (5)

^I Race specific percentiles