CCL2 Polymorphisms Are Associated With Serum Monocyte Chemoattractant Protein-1 Levels and Myocardial Infarction in the Framingham Heart Study

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Background—Monocyte chemoattractant protein-1 (MCP-1) is a chemokine strongly implicated in promoting atherosclerosis in animal models, but human genetic evidence is contradictory.

Methods and Results—We analyzed the association of genetic variation in the MCP-1 gene (*CCL2*) with prevalent myocardial infarction and serum MCP-1 levels in the community-based Framingham Heart Study Offspring Cohort (50% women; mean age, 62 years). MCP-1 levels and *CCL2* genotypes were determined in 3236 and 1797 individuals, respectively. Significant clinical correlates of MCP-1 levels were age, cigarette smoking, triglycerides, body mass index, and waist-to-hip ratio. The *MCP-1-2578G* allele located in the *CCL2* regulatory region was significantly associated with both higher serum MCP-1 levels in a recessive genetic model (358±10 versus 328±3 pg/mL; $P=0.002$) and higher prevalence of myocardial infarction in a dominant genetic model (adjusted odds ratio, 2.0; 95% CI, 1.2 to 3.3; $P=0.005$). We also defined the linkage disequilibrium structure at the *CCL2* locus and observed 6 common haplotypes in whites. We performed haplotype-based association analysis and found that only the most frequent haplotype, defined by the *MCP-1-2578G* allele, was associated with prevalent MI.

Conclusions—Our data are consistent with the hypothesis that MCP-1 is involved in the pathogenesis of human atherosclerosis and myocardial infarction. (***Circulation*. 2005;112:1113-1120.**)

Key Words: epidemiology ■ genetics ■ inflammation ■ myocardial infarction ■ risk factors

Atherosclerosis is now recognized as a disease of arterial inflammation that arises from the interactions of migratory leukocytes with resident vascular endothelial cells, smooth muscle cells, and fibroblasts.[1] At the molecular level, interactions among these cell types are regulated by cytokines, adhesion molecules, and chemoattractants. Chemokines are a large family of chemoattractants that direct migration of leukocytes from the blood to sites of inflammation.[2]

Several lines of evidence suggest that the chemokine, monocyte chemoattractant protein-1 (MCP-1; gene name, *CCL2,* previously *SCYA2*) and its receptor CCR2 (gene name, *CCR2*) are involved in atherosclerosis.[3] In various murine models of atherosclerosis (apolipoprotein E and LDL receptor knockouts, apolipoprotein B transgenics), deletion of *CCL2* or *CCR2* results in large (50% to 80%) reductions in atherosclerotic plaque size.[4–6] Conversely, overexpression of MCP-1 in the leukocytes of susceptible mice results in increased plaque size.[7] Furthermore, in animal models, temporary blockade of CCR2 by antibodies or gene therapy blocks restenosis after balloon angioplasty and stabilizes and reduces atherosclerotic lesion size.[8–10]

MCP-1 is expressed in human atherosclerotic lesions, and CCR2 is expressed on leukocytes.[11–13] In addition, MCP-1 induces arrest and transmigration from the circulation of CCR2+ monocytes under conditions of physiological shear force and promotes monocyte differentiation to lipid-laden macrophages.[14,15] MCP-1 also contributes to the proliferation of arterial smooth muscle cells,[16] which, along with macrophages, constitute the key cellular components of atherosclerotic plaques.

A growing number of human epidemiological studies have suggested links between circulating MCP-1 levels and ath-

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erosclerosis. Higher MCP-1 levels have been associated with increased risks of myocardial infarction (MI), sudden death, coronary angioplasty, and stent restenosis.[17–20] However, little is known about the role of MCP-1 levels in cardiovascular disease (CVD) in the general population.

Genetic variation of *CCL2* and *CCR2* could modulate MCP-1 production and function. In fact, 3 studies have associated the *CCL2* distal regulatory region single nucleotide polymorphism (SNP) *MCP-1-2578G* with increased circulating levels of MCP-1.[21–23] However, genetic associations reported between *MCP-1-2578G* and CVD end points have not been consistent. Two case-control studies have reported an association between *MCP-1-2578G* and atherosclerosis in whites. The first reported an increased frequency of *MCP-1-2578G* homozygotes (odds ratio [OR], 2.2; $P<0.005$) among individuals referred for CABG compared with age- and sex-matched controls (n=638).[24] The second found an increased frequency of the *MCP-1-2578G* allele among HIV patients with subclinical atherosclerosis identified by ultrasound (OR, 5.7; $P=0.004$) compared with those lacking the allele (n=183).[25] However, a third study was unable to find an association between the same polymorphism and MI in a Japanese cohort (n=909),[26] and a fourth study found no significant association of this allele with angiographically determined coronary artery disease (CAD) in whites (n=3224).[27] The inconsistency of these results could be due in part to the case-control design of these studies or to reliance on the testing of a single polymorphism. To address these issues, we have conducted an analysis of *CCL2* genetic variation and its relation to serum levels of MCP-1 and prevalent MI in the Framingham Heart Study (FHS) Offspring Cohort, a large, longitudinal, observational, community-based study of the epidemiology of CVD.

Methods

FHS Offspring Cohort Subjects

The design of the FHS has previously been described.[28,29] Briefly, the FHS enrolled 5124 children of the original participants and their spouses, referred to as the Offspring Cohort, beginning in 1971. Participants undergo a routine comprehensive medical examination every ≈4 years. At the cohort's seventh examination cycle, 3236 attendees had serum MCP-1 levels (henceforth referred to as MCP-1 levels) measured. The FHS Offspring Cohort contained 1888 unrelated participants for whom DNA was available in 1995 to 1998. Of these, 1797 gave informed consent for genotyping, had clinical and genotype information available, and were included in the genotype-phenotype analyses; MCP-1 levels were performed in 1602 genotyped participants. The study was approved by the National Institute of Allergy and Infectious Diseases and Boston University School of Medicine Institutional Review boards, and all participants signed informed consent.

Clinical Risk Factors and Cardiovascular Outcomes

Outcomes were evaluated up to the time of the seventh examination (2002, the last year for which data were complete) by investigators blinded to genotypic data. MI was defined as either hospitalization for acute MI (≈95%) or as new ECG evidence consistent with a clinically unrecognized MI (≈5%). The occurrence of MI was determined by a 3-physician end-point committee that reviewed records from routine study examinations outside physicians' offices and hospitalizations. Of the subjects whose *CCL2* genotypes had been determined, 107 (6%) had prevalent MI at the time of the seventh examination. CVD risk factors, including serum total cholesterol, HDL, and triglycerides, were classified at the seventh examination cycle. Smoking was defined as cigarette use within the prior year. Diabetes was defined as fasting blood glucose level ≥7.0 nmol/L (126 mg/dL) or treatment with hypoglycemic agents. Hypertension was defined as systolic or diastolic blood pressure ≥140/90 mm Hg or antihypertensive medication treatment. Use of medications in the statin or β-blocker classes was also evaluated as 2 separate dichotomous variables (yes/no) at the time of the seventh examination cycle.

Measurement of Serum MCP-1 Levels

Fasting serum samples were collected at the seventh examination and stored at −70°C. For analysis, samples were thawed and vortexed vigorously, and MCP-1 levels (pg/mL) were measured in duplicate according to the manufacturer's instructions with a commercially available ELISA (R&D Systems). All samples with an intra-assay coefficient of variation above the 95th percentile were repeated; the mean of repeated duplicate values was used. Initially, samples had an average coefficient of variation of 6.1±3.3%; samples with a coefficient of variation >10.9% were rerun. However, we improved our pipetting technique. For the final 43% of samples, we achieved an average coefficient of variation of 1.9±1.6%; samples with a coefficient of variation >5.3% were rerun.

Haplotype Block Definition and Selection of Haplotype Tag SNPs

From the public dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), we selected 24 evenly spaced markers within a 10.6-kb region spanning the *CCL2* gene (GenBank NM_002982). The SNPs were genotyped in a panel of 12 multigenerational white family pedigrees from the Centre d'Etude du Polymorphisme Humain (CEPH) Utah panel (Coriell Institute for Medical Research, Camden, NJ).[30] These reference pedigrees included 93 individuals representing 96 independent chromosomes of European ancestry. Assays were considered successful if they met the following criteria: at least 75% success for genotyping calls, Hardy-Weinberg equilibrium $P>0.01$, and Mendelian transmission errors ≤1. In addition, we imposed a minor allele frequency threshold and defined "common" for the present study as a minor allele frequency ≥5%. Overall, we developed successful assays for 9 SNPs.

Haplotype blocks were defined through the use of the criteria of Gabriel et al,[30] and tag SNPs were selected in the publicly available Haploview software package, version 2.03.[31] For each pair of markers, we calculated D′, an estimate of the strength of linkage disequilibrium [LD], and a logarithm of the odds (LOD) score, which is an estimate of LD significance. From these 2 measures, each pairwise marker comparison was categorized into 1 of 3 groups: (1) no or minimal evidence of historical recombination (D′=1/LOD >2 or 0.5<D′<1/LOD >2), (2) strong evidence of historical recombination (D′<1/LOD <2 or D′<0.5/any LOD), and (3) uninformative (D′=1.0/LOD <2).

Genotyping

CCL2 genotyping was performed on coded DNA samples by laboratory personnel blinded to clinical information. The genotyped variants were named to reflect the distance in nucleotides from the translational start point in GenBank D26087 and their unique identifiers in the NCBI SNP database (dbSNP; http://www.ncbi.nlm.nih.gov/SNP). We used previously published methods for the *MCP-1-2578* and *MCP-1-2136* polymorphisms (also previously called *MCP-1-2518* and *MCP-1-2076*).[21] In addition to these 2 previously studied SNPs, 5 tag SNPs were genotyped with commercially available (Sequenom) matrix-associated laser desorption ionization time-of-flight mass spectrometry technology and previously published protocols.[30]

Statistical Analysis

MCP-1 levels were logarithmically transformed before analysis because they were not normally distributed. Covariate selection for logarithmically transformed MCP-1 levels was conducted with a

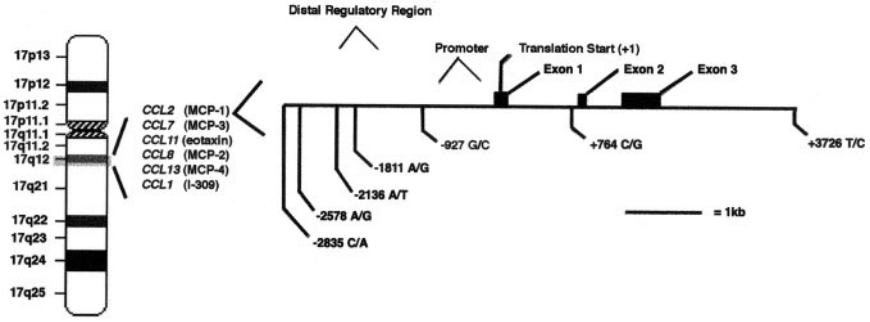

Figure 1. Chromosomal location, genomic organization, and location of polymorphic variation in *CCL2* gene. *CCL2* lies on long arm of chromosome 17 in cluster of other chemokine genes. It has 3 exons extending over ≈2000 bp. It is known to have both distal and proximal regulatory elements important for cytokine-induced and constitutive activity, respectively. Polymorphisms studied here span gene and are named for their distance in nucleotides from translational start point.

SOLAR regression model to account for correlation among members of the same family. In the SOLAR regression model, the correlation between any pair of relatives was assumed to be proportional to their kinship coefficient.[32] The significance of each covariate was evaluated after adjustment for all the other factors in a likelihood ratio test. Covariates with a value of $P<0.05$ were kept in the final model (age and sex were forced in), selecting from the following: ratio of total to HDL cholesterol, triglycerides, systolic and diastolic blood pressures, body mass index, waist-to-hip ratio, fasting glucose, diabetes, hypertension treatment, lipid treatment, smoking status, and alcohol consumption. Regression coefficients were expressed by presence (versus absence) for dichotomous variables and per-unit increase for continuous variables. Unadjusted analyses of individual *CCL2* genotypes with prevalent MI were performed by cross-tabulation and calculation of ORs. Multivariable-adjusted logistic regression analyses were conducted to examine the partial contribution of *CCL2* genotype to risk of prevalent MI (SAS, version 8, SAS Institute Inc).[33] The OR and 95% confidence limits were calculated with this model; significance was evaluated based on Wald χ^2 tests. The association between established risk factors and *CCL2* genotypes was examined by a χ^2 test with 1 *df* for dichotomous measurements and by testing the differences in means of the risk factors between *CCL2* genotype groups with a 2-tailed Student *t* test for continuous measurements. Tests of interaction between *CCL2* genotypes and the established risk factors were also performed using logistic regression, in which 2-*df* Wald χ^2 tests were performed for the interactions between the *CCL2* genotype and age, sex, body mass index, hypertension, diabetes, and smoking. Haplotype-based association analyses were conducted using a weighted regression approach as implemented in the haplo.score program.[34,35] Haplotype frequency was estimated with the expectation-maximization algorithm.[36] All compatible haplotype configurations of a multimarker genotype were used in the regression, with weights being the corresponding likelihood of such configuration. A global score statistic tested all haplotypes simultaneously to detect any departure from the null hypothesis of no association.

TABLE 1. Clinical Characteristics of Participants With MCP-1 Levels and Genotype Data

Risk Factors	With MCP-1 Level (n=3236)	Genotyped (n=1797)
Mean age, y	61±9	62±9
Male sex, %	53.0	50.0
Hypertension, %	45.5	47.7
Diabetes, %	13.3	15.4
Cigarette smoking, %	12.9	13.0
Alcohol consumption, oz/wk	2.6±3.8	2.7±3.8
Body mass index, kg/m^2	28.1±5.3	28.1±5.2
HDL cholesterol, mg/dL	54±17	53±17
Total cholesterol, mg/dL	200±37	200±36
Triglycerides, mg/dL	137±89	136±87
Use of statins, %	18.9	19.5
Use of β-blockers, %	17.2	18.8
Estrogen therapy, % women	32.2	33.5
Menopause, % women	86.8	88.9
Waist-to-hip ratio	1.0±0.1	1.0±0.1
Prevalent MI, %	5.4	6.0
MCP-1 levels, pg/mL	328±124	330±123

Results

We assessed MCP-1 levels in 3236 participants and genotyped 7 common *CCL2* SNPs spanning the gene (Figure 1) in 1797 unrelated participants of the FHS. Table 1 displays the clinical characteristics of participants with MCP-1 levels and those with genotypes.

Clinical Correlates of MCP-1 Levels

In stepwise linear regression models, the clinical correlates of MCP-1 levels were age, cigarette smoking, triglycerides, body mass index, and waist-to-hip ratio (Table 2). Together, these covariates explained only 6% of the variability in MCP-1 levels. MCP-1 levels were not associated with prevalent MI in sex-specific or sex-pooled multivariable models (OR, 1.0; 95% CI, 0.99 to 1.00; $P=0.50$).

CCL2 Genotypes in the FHS Offspring Cohort

We found the *MCP-1-2578* and *MCP-1-2136* allele frequencies to be similar to those reported previously (30% and 21%, respectively, in a previous study versus 27% and 21% in the

TABLE 2. Clinical Correlates of Logarithm-Transformed Serum MCP-1 Levels

Clinical Factors	Regression Coefficient*	*P*
Age (per 10 y)	0.07	<0.001
Sex (male vs female)	−0.008	0.86
Cigarette smoking (present)	0.09	<0.001
Triglycerides (mg/dL)	0.0002	0.0007
Body mass index (kg/m^2)	0.003	0.013
Waist-to-hip ratio	0.22	0.02

*Regression coefficients are expressed for dichotomous variables as presence vs absence and for continuous variables per unit increase for variables retained in final stepwise models (age and sex forced in). Alcohol consumption, lipid treatment, fasting glucose, diabetes, total/HDL cholesterol, hypertension treatment, and systolic and diastolic blood pressures were not retained in the final model.

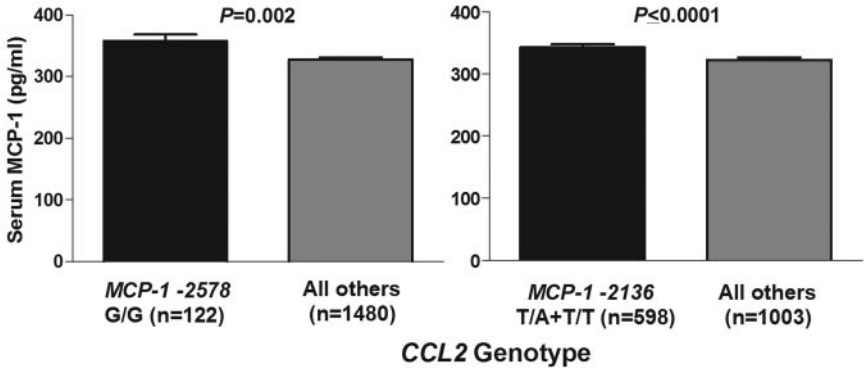

Figure 2. Serum MCP-1 levels vary according to *CCL2* genotype. Mean±SEM values for samples obtained at seventh examination cycle are given for indicated genotypic groups.

present study).[21] Also consistent with previous studies, in our subjects, these 2 polymorphisms were in complete LD with each other. The genotype frequencies for each allele considered separately were consistent with Hardy-Weinberg predictions.

MCP-1 Genotypes and Serum MCP-1 Levels

A previous association has been demonstrated between MCP-1-2578G and unstimulated MCP-1 levels.[21–23] Therefore, we examined the association in participants with available MCP-1 levels and genotypes (n=1602, 89% of those genotyped). We found a significant positive correlation of MCP-1 levels with both the MCP-1-2578G allele in a recessive genetic model and the MCP-1-2136T allele in a dominant genetic model (Figure 2). After multivariable regression adjusting for variables found to be significantly associated with MCP-1 level (Table 2), both associations remained significant ($P=0.002$ and $P\leq0.0001$, respectively).

CCL2 Genotypes and Prevalent MI

In a dominant genetic model, possession of an *MCP-1-2578G* allele was marginally associated with increased MI prevalence in the cohort as a whole (unadjusted OR, 1.5; 95% CI, 1.0 to 2.2; $P=0.06$; Table 3). In men, the association appeared stronger (unadjusted OR, 1.8; 95% CI, 1.2 to 2.9; $P=0.009$). There were no significant differences in established risk factors in those with or without an *MCP-1-2578G* allele. After multivariable adjustment for the covariates listed in Table 3, the *MCP-1-2578G* allele was associated with prevalent MI in the sex-pooled cohort (OR, 2.0; 95% CI, 1.2 to 3.3; $P=0.005$). In tests for effect modification, there were no significant interactions between age, sex, diabetes mellitus, cigarette smoking, or hypertension and either of the 2 polymorphisms with regard to MI prevalence. The multivariable-adjusted OR was strong in men (OR, 2.6; 95% CI, 1.5 to 4.5; $P=0.0006$), whereas the association with increased prevalent MI was not found in women (OR, 0.7; 95% CI, 0.2 to 2.2; $P=0.50$). However, very few women had prevalent MI (n=23), and there was insufficient power (0.60) to exclude a significant association in women compared with men.

In multivariable models incorporating MCP-1 levels as a covariate, possession of the *MCP-1-2578G* allele continued to be significantly associated with increased MI prevalence (OR, 1.9; 95% CI, 1.2 to 3.2; $P=0.009$; Table 3). Hence, measurement of unstimulated MCP-1 level at 1 time point did not fully account for the association between *MCP-1-2578G* genotype and MI prevalence.

CCL2 Haplotype Analysis

To more fully understand the extent of genetic variation at the *CCL2* gene, we studied the haplotype block structure in

TABLE 3. Prevalence of MI According to *CCL2* Genotype for Men and Women Pooled and Separately

			Unadjusted		Multivariable Adjustment		Multivariable Adjustment and MCP-1 Levels	
CCL2 Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	*P*	OR (95% CI)	*P**	OR (95% CI)	*P*†
Pooled								
MCP-1–2578 G/G+G/A	60 (3)	787 (44)	1.5 (1.0–2.2)	0.06	2.0 (1.2–3.3)	0.005	1.9 (1.2–3.2)	0.009
All others	47 (3)	902 (50)	1.0	...	1.0	...	1.0	...
Male								
MCP-1–2578 G/G+G/A	50 (6)	362 (40)	1.8 (1.2–2.9)	0.009	2.6 (1.5–4.5)	<0.001	2.5 (1.4–4.5)	0.001
All others	34 (4)	453 (50)	1.0	...	1.0	...	1.0	...
Female								
MCP-1–2578 G/G+G/A	10 (1)	425 (47)	0.8 (0.4–1.9)	0.63	0.7 (0.2–2.2)	0.50	0.7 (0.2–2.3)	0.54
All others	13 (1)	449 (50)	1.0	...	1.0	...	1.0	...

*Multivariable model adjusted for age, sex, diabetes, hypertension, body mass index, alcohol use, smoking, HDL and total cholesterol, triglycerides, and use of β-blockers or statins.

†Multivariable adjustment and MCP-1 level model additionally adjusted for measured serum MCP-1 level at exam 7.

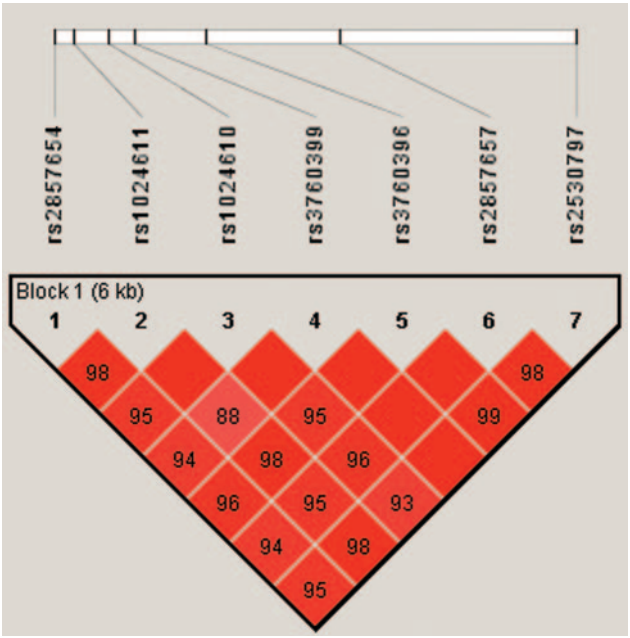

Figure 3. Linkage disequilibrium (LD) structure at *CCL2* locus in the Framingham Heart Study sample. Single haplotype block encompassed *CCL2* gene. LD structure for 7 common single nucleotide polymorphisms labeled by their unique RS numbers vertically and 1 to 7 horizontally spanning 6.5 kb across *CCL2* gene is shown. Note SNP 1 is *MCP-1-2835 C/A*, 2 is *MCP-1-2578 A/G*, 3 is *MCP-1-2136 A/T*, 4 is *MCP-1-1811A/G*, 5 is *MCP-1-927 G/C*, 6 is *MCP-1+764 C/G*, and 7 is *MCP-1+3726 T/C*. Each square denotes strength and significance of LD between pairs of markers in region. Red indicates no or minimal evidence of historical recombination. Numbers in squares indicate 100× D′, statistical measure of LD, with missing values indicating result of 100.

CEPH reference pedigrees, identified 5 additional SNPs that tagged common haplotypes, and genotyped them in the participants. Using all the genetic information, we then performed a haplotype-based association analysis of both MCP-1 level and prevalent MI. We found that the *CCL2* gene was encompassed in 1 haplotype block in both the CEPH pedigrees and the FHS participants (Figure 3), with nearly identical allele and haplotype frequencies. Seven *CCL2* SNPs delineated 6 common haplotypes (H1 through H6) that accounted for 97% of all haplotypes in the FHS (Table 4). We observed that 2 of the additional SNPs (*MCP-1-2835A* and *MCP-1+764G*) were at similar frequency and were in complete LD with the previously studied SNPs (*MCP-1-2578G* and *MCP-1-2136T*, respectively). *CCL2* haplotype 2 (H2), which was defined by *MCP-1-2136T or MCP-1+764G*, was significantly associated with MCP-1 level before and after adjustment ($P=0.005$) for the relevant clinical factors. None of the other 5 haplotypes was significantly associated with MCP-1 levels in this additive model. *CCL2* haplotype 1 (H1), which was defined by *MCP-1-2578G* or *MCP-1-2583A*, was significantly associated with prevalent MI in men after adjustment for the relevant clinical factors, including MCP-1 levels ($P<0.01$). No other haplotype was significantly associated with prevalent MI. Results of association analyses of each of the 7 SNPs individually with serum MCP-1 level and prevalent MI are shown in Table 5. The *MCP-1-2835A* SNP, in high LD with the previously studied *MCP-1-2578G* SNP and carried on H1, was also associated with both prevalent MI and serum MCP-1 levels. The *MCP-1+764G* SNP in high LD with *MCP-1 to 2136T* and carried on H2 also was associated with MCP-1 levels at a similar level of statistical significance.

Discussion

This study provides evidence from a large, community-based cohort supporting the hypothesis that the chemokine MCP-1 is a pathogenic factor in human CVD. First, we found that homozygotes for the *MCP-1-2578G* allele had higher basal unstimulated MCP-1 levels. This result is consistent with previous findings in much smaller studies that the *MCP-1-2578G* allele is associated with increased circulating levels of MCP-1.[21–23] Second, we made the novel observation that individuals possessing the *MCP-1-2136T* allele also had higher MCP-1 levels. Both of these genetic associations remained highly significant after adjustment for other clinical factors associated with MCP-1 levels. Third, possession of the *MCP-1-2578G* allele was associated with an increased risk of prevalent MI. This result is consistent with those of 2 previously published case-control studies that reported an association of the *MCP-1-2578G* allele with an increased risk of atherosclerosis.[24,25] Furthermore, we have performed the first comprehensive analysis of the common genetic variation of *CCL2* in a large community-based population and identified novel variants that are in strong LD with *MCP-1-2578G* (*MCP-1-2835A*) and *MCP-1-2136T* (*MCP-1+764G*).

The *MCP-1-2578G* association with MI persisted after adjustment for other well-established risk factors and was similar in magnitude to that previously reported for angiographically proven CAD.[24] Moreover, the magnitude of

TABLE 4. CCL2 Polymorphisms and Resultant Inferred Haplotype Frequencies

	Names of SNPs Tested and Nucleotides Detected								
Gene position	−2835	−2578	−2136	−1811	−927	+764	+3726		
Unique RS No.	2857654	1024611	1024610	3760399	3760396	2857657	2530797	Haplotype	Frequency, %
	A	G	A	A	G	C	T	H1	27.0
	C	A	T	A	G	G	C	H2	20.3
	C	A	A	A	C	C	T	H3	18.6
	C	A	A	A	G	C	C	H4	18.3
	C	A	A	A	G	C	T	H5	8.6
	C	A	A	G	G	C	T	H6	4.2

TABLE 5. Association of Individual *CCL2* Allelic Variants and Serum MCP-1 Levels in Men and Women Pooled and MI in Men

Allelic Variant				Genotyping	MCP-1 Level		MI	
Gene Position	Unique RS No.	Variant Type	MAF	Success, %	*P**	*P*†	*P*‡	*P*§
−2835	2857654	DRR	0.29	94	0.01	0.004	0.009	0.0008
−2578	1024611	DRR	0.27	100	0.001	0.0005	0.02	0.0035
−2136	1024610	DRR	0.21	100	0.0004	0.0005	0.28	0.09
−1811	3760399	DRR	0.04	96	0.49	0.38	0.13	0.32
−927	3760396	Promoter	0.19	95	0.03	0.08	0.75	0.54
+764	2857657	First intron	0.21	98	0.0007	0.002	0.56	0.12
+3726	2530797	3′ Flanking	0.40	99	0.34	0.44	0.36	0.13

MAF indicates minor allele frequency; DRR, distal regulatory region.

*Unadjusted, †multivariable adjusted for covariates in Table 2; global *P* value in Framingham men and women with levels (n=1602).

‡Unadjusted, §multivariable adjusted for covariates in Table 3, including MCP-1 level; global *P* value in Framingham men (n=899).

increased risk associated with possession of the *MCP-1-2578G* allele was similar to the magnitude of risk augmentation attributable to established MI risk factors. For example, in the FHS Offspring Cohort, the multivariable-adjusted ORs for prevalent MI were 2.5 for diabetes, 2.2 for recent smoking, and 4.9 for male sex compared with 2.0, the OR for possession of the *MCP-1-2578G* allele compared with all others in this cohort (Table 3). Together, these data suggest a pathogenetic role for MCP-1 in human atherosclerotic CVD.

The exact mechanism by which the *MCP-1-2578G* allele might increase MI risk remains uncertain; however, presumably locally increased MCP-1 levels in the coronary arteries would promote transmigration of CCR2+ leukocytes from the blood and their organization and activation in plaque. Consistent with this model, MCP-1, CCR2, and activated macrophages are found in atherosclerotic lesions.[11–13] In this model of pathogenesis, the interaction between MCP-1 and CCR2 may play several roles, but it is probably not the only factor involved in lesion formation because lesions are able to form in the absence of either of these molecules in susceptible animal models.[4–6] It should be noted that CCR2 is also polymorphic in humans, and in a previous study, we investigated the association in the Framingham Offspring Cohort of an SNP of unknown functional significance in the CCR2 receptor, *CCR2-64I*. This SNP results in the conservative replacement of a valine at position number 64 with an isoleucine. We found a nonsignificant association of this SNP with CVD using a dominant genetic model (OR, 0.7; $P=0.12$) after multivariable adjustment.[37] In the present study, the association of *MCP-1-2578G* with prevalent MI was not substantially altered by incorporating the baseline MCP-1 level as a covariate. One potential explanation is that baseline unstimulated MCP-1 levels may not reflect local tissue levels in the coronary arteries where inflammatory cytokines like interleukin-1 and tumor necrosis factor are known to be expressed.[38] Previous in vitro studies have found these cytokines to have marked effects on the regulatory region where the *CCL2* SNPs associated with CVD are located.[39,40] In addition, the measured level of MCP-1 was done only at 1 time point, whereas longitudinal levels may be more important.

We noted that the clinical correlates of MCP-1 levels in the community were age, cigarette smoking, triglycerides, body mass index, and waist-to-hip ratio but that these factors explained only 6% of the variability in MCP-1 levels. Genetic factors may play a more important role in MCP-1 level variability. In a separate study, we have recently noted that the adjusted heritability of MCP-1 levels is 44% and found significant linkage on chromosome 1 at 186 cM (LOD, 4.27; genome-wide $P=0.005$).[41] In the Oral Glycoprotein IIb/IIIa Inhibition with Orbofiban in Patients With Unstable Coronary Syndromes (OPUS-TIMI) 16 trial, MCP-1 levels were related to advancing age, female sex, hypertension, diabetes, and renal insufficiency.[17] We suspect that de Lemos and colleagues[17] observed different correlates because of the referral nature of their cohort. The relation of MCP-1 levels with 2 measures of adiposity in our cohort is of interest because of the observations by Christiansen et al[42] that adipocyte MCP-1 mRNA levels correlated with circulating MCP-1 and body mass index and that weight loss was associated with a decline in circulating MCP-1 levels.

Study Strengths and Limitations

FHS is a large community-based cohort, diminishing referral biases, with routine ascertainment of CVD risk factors and events, enabling multivariable analyses and genotyping and phenotyping blinded to one another. The observational, nonrandomized nature of cardiovascular medications indicates that caution should be observed with regard to the lack of association between statins and other treatments and MCP-1 levels. Initially, we performed relatively few statistical tests to confirm the 2 previously reported SNP associations.[24,25] However, we augmented our analyses with haplotypes and marker levels to examine more comprehensively the relation of MCP-1 and *CCL2* to risk factors and disease. Hence, we have performed multiple statistical tests that increase the possibility of false-positive associations. Furthermore, our study examined prevalent MI; an apparent association for the *MCP-1-2578G* allele might represent a survival bias if individuals with that allele had a lower case fatality rate than other genotypes. Because we had few prevalent MI cases in women, we were underpowered to find an association be-

tween the *CCL2* genotypes and MI in women. Additional follow-up of our sample or investigation of larger samples of women will help to clarify whether the risk of MI associated with *CCL2* genotype varies by gender. Our study sample was largely middle-aged to elderly and white; the findings may not be generalizable to younger individuals or other racial groups. However, the single-race and community-based structure of the cohort minimizes the possibility that the associations reported here were due to population stratification. Although our results are consistent with 2 previous studies relating *MCP-1-2578G* to atherosclerosis phenotypes (CAD and carotid intima-media thickness),[24,25] our report is the first to relate *MCP-1-2578G* to the MI phenotype.

Because of the tight LD between *MCP-1-2835A* and *MCP-1-2578G* on haplotype H1 and similarly between *MCP-1-2136T* and *MCP-1+764G* on haplotype H2, it is very difficult to separate their influence in our genetic association study. Association studies in populations with greater haplotypic diversity (eg, populations of African ancestry) and functional studies involving the newly identified sites (*MCP-1-2835A* for MCP-1 levels/MI and *MCP-1+764G* for MCP-1 levels) may help to clarify which, if any, affects production of MCP-1. In addition, more comprehensive resequencing is needed to look for other functional sites in the relevant haplotypes. Whereas it remains possible that the association is due to linkage with another as-yet unknown polymorphism, the evidence that *MCP-1-2578G* is a functional polymorphism using in vitro promoter assays supports the hypothesis that there is a functional role for this SNP.[39] Electromobility gel shift assays have also shown allele-specific nuclear factor binding to oligonucleotide probes corresponding to the *MCP-1-2578G* site from TNF-stimulated hepatic primary cells and MG-63 osteosarcoma cells.[21,40] The identity of these induced nuclear factors is not yet completely known but at least in MG-63 cells appeared to contain interferon regulatory factor-1.[21]

Research and Clinical Implications

Our data suggest several future research directions such as the investigation of the identity of the functional SNP(s) in these haplotypes, whether these SNPs act alone or instead require interaction with other SNPs, and whether transcription factors bind at these polymorphic sites in leukocytes and endothelial cells. In addition, the *CCL2* polymorphisms we examined may be generally useful as genetic probes to evaluate the potential role of MCP-1 in the pathogenesis of other inflammatory diseases in humans.

We observed that the *MCP-1-2578G* allele is dominantly associated with increased risk of MI in humans after adjustment for other CVD risk factors. Our results provide additional support for the inflammation hypothesis of atherosclerosis pathogenesis and for further study to determine the role of MCP-1 as a proinflammatory risk factor. Identifying inflammatory polymorphisms contributing to atherosclerosis will improve our understanding of the pathogenesis of CVD and may suggest targets for improved prevention and treatment strategies.

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