Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2009;28:124–130
DOI: 10.1159/000223437

Received: September 15, 2008
Accepted: March 30, 2009
Published online: June 5, 2009

Single Nucleotide Polymorphisms in Monocyte Chemoattractant Protein-1 and Its Receptor Act Synergistically to Increase the Risk of Carotid Atherosclerosis

Paul A. Nyquist[a] Cherie A. Winkler[c] Louise M. McKenzie[c] Lisa R. Yanek[b]
Lewis C. Becker[b] Diane M. Becker[b]

[a]Departments of Anesthesiology/Critical Care Medicine, Neurology, and Neurological Surgery, Johns Hopkins School of Medicine, and [b]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Md., and [c]National Cancer Institute, National Institutes of Health, Frederick, Md., USA

Key Words
Carotid atherosclerosis · Monocyte chemoattractant protein · Single nucleotide polymorphisms · Inflammation

Abstract
Background: Monocyte chemoattractant protein 1 (MCP-1), acting in concert with its receptor chemokine receptor 2 (CCR2), promotes recruitment of macrophages into atherosclerotic plaque. We examined whether single nucleotide polymorphism (SNP) variants in the MCP-1 or CCR2 genes independently or in combination are associated with carotid artery atherosclerosis in an African American population at increased risk of vascular disease. ***Methods:*** Four SNPs in MCP-1 and 1 in CCR2 were genotyped. Carotid artery duplex ultrasonography was used to identify the presence or absence of carotid plaque >1 mm. The study population included 325 apparently healthy 30- to 59-year-old black siblings of 185 probands with premature coronary artery disease (<60 years old). Associations between each independent SNP and the presence of carotid plaque were examined using multivariate logistic regression models adjusted for age, sex, educational level, diabetes, smoking, hypertension, obesity, low-density lipoprotein cholesterol and non-independence within families. Interactions between SNPs in the MCP-1 gene and the SNP in the CCR2 gene were examined by multivariate analysis. ***Results:*** Siblings were 32% males, with a mean age of 46 ± 7 years, and 77 (24%) demonstrated carotid plaque. In multivariate analyses, the CC genotype of MCP-1 SNP rs2857656 was independently associated with plaque (p = 0.05). Subjects who had both the MCP-1 CC genotype and were heterozygotic or homozygotic for the CCR2 V64I genotype (rs1799864; n = 12) had an even higher risk of carotid atherosclerosis (odds ratio 6.14, 95% confidence interval 1.82–20.73; p = 0.0037). ***Conclusion:*** The MCP-1 rs2857656 CC genotype is independently associated with carotid artery plaque in African American from families with premature coronary artery disease. The combination of the MCP-1 CC homozygous genotype and the homozygotic or heterozygote CCR2 V64I genotype is associated with a particularly high prevalence of carotid artery plaque.

Background and Purpose

Carotid atherosclerosis is a chronic inflammatory disease. Evidence suggests that functional polymorphisms in genes that regulate inflammation may play a significant role in the development of atherosclerosis [1–10].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ced

Paul A. Nyquist, MD, MPH
Departments of Anesthesiology/Critical Care Medicine, Neurology, and Neurological Surgery, Johns Hopkins School of Medicine
Meyer 8-140, 600 N. Wolfe St., Baltimore, MD 21287 (USA)
Tel. +1 410 955 2611, Fax +1 410 614 7903, E-Mail pnyquis1@jhmi.edu

Monocyte chemoattractant protein 1 (MCP-1) and its receptor chemokine receptor 2 (CCR2) are proinflammatory molecules that play an important role in the early recruitment of monocytes following injury to the vascular wall and contribute to the development of atherosclerosis [6, 10, 11–16]. The MCP-1 –2578 G/A single nucleotide polymorphism (SNP) has been associated with coronary artery disease (CAD) in studies in the Framingham cohort, a Tunisian population, and in our own study population [4–6]. Recently, an SNP causing an isoleucine-for-valine substitution at position 64 in the CCR2 receptor (CCR2, rs1799864) on chromosome 3 has been associated with ischemic cardiomyopathy and myocardial infarction in Czech populations [7, 8]. As yet, no study has identified any epistasis or synergy between SNPs in the MCP-1 and CCR2 genes and atherosclerotic disease [17], nor have any SNPs in these genes been associated with a risk of carotid atherosclerosis.

Begun in 1983, the Johns Hopkins Sibling and Family Heart Study is a prospective study of the environmental and genetic causes of premature cardiovascular disease. Almost 900 probands have been identified with documented CAD before age 60, and the study has enrolled their healthy siblings (aged 30–59 years) for risk factor screening.

It was our goal to examine the relationship between (1) selected SNPs in the MCP-1 gene and its receptor CCR2, and (2) carotid plaque as identified by carotid ultrasound (CUS). We also examined the issue of any synergy between the well-characterized rs1799864 SNP in the CCR2 (rs1799864) gene and any SNPs in the MCP-1 gene in our black population.

Materials and Methods

Participants were screened in the Johns Hopkins Sibling and Family Heart Study. The study population was restricted to the healthy siblings of the probands who had CAD before the age of 60. The probands themselves were not included. All study participants were asymptomatic for any type of vascular disease. They included 325 African Americans who had undergone a CUS study and had contributed a blood sample for DNA. They were selected for genotyping of the MCP-1 and CCR-2 genes. All subjects were asymptomatic, without clinical signs of CAD or stroke and provided informed consent prior to any measurements.

Demographic information (age, sex, race, education) was self-reported by participants. Height and weight were measured, and the body mass index was calculated as weight (kg)/height (m^2). Obesity was determined through comparison with published standards [18]. All lipid and glucose values were obtained in the fasting state and were measured in a Clinical Laboratory Improvement Amendments-approved laboratory. Blood pressure was measured 3 times during the screening day, and the average of these readings was used in analyses. Hypertension was defined as an average blood pressure ≥140/90 mm Hg and/or use of antihypertensive medication. Diabetes was defined as glucose >6.94 mmol/l (125 mg/dl) and/or use of insulin or oral medication. Smoking status was self-reported and confirmed by exhaled carbon monoxide (≥8 defined a current smoker). All biometric data were obtained concurrently with the CUS.

All DNA was extracted from whole blood and purified with a 'Qiagen Kit,' using standard techniques. The DNA was frozen at –70°C until use. The following SNPs were genotyped in MCP-1: –2578 A/G (rs1024611), –2136 A/T (rs1024610), –362 G/C (rs2857656) and 903 C/T (rs4586). The V64I CCR2 (rs1799864) SNP was also genotyped. Genotyping was performed using TaqMan assays according to the manufacturer's instructions (Applied Biosystems, Foster City, Calif., USA) at the laboratories of the National Cancer Institute, using established protocols and probes (available upon request). No significant errors were detected in the repeat assay. All assays were performed under the supervision of Cheryl Winkler. Occult carotid atherosclerosis was identified by high-resolution extracranial CUS. Atherosclerotic plaques were defined visually as a focal encroachment into the vascular lumen of >1.0 mm in predefined areas (right distal common carotid artery, left distal common carotid artery, right bulb, left bulb, right proximal internal carotid artery, left proximal internal carotid artery). Imaging was performed by 2-dimensional B-mode ultrasound (Sonosite MicroMaxx ultrasound machine, 13-6 MHz linear probe). All measurements were completed by a trained ultrasonographer using digital calipers.

Study Design and Data Analysis

Three SNPs genotyped in the MCP-1 gene were chosen because of their position within the MCP-1 promoter region [–2578 A/G (rs1024611), –2136 A/T (rs1024610) and –362 G/C (rs2857656)]. These SNPs were thought most likely to have an influence on the transcriptional activity of MCP-1. Two (–2578 A/G and –2136 A/T) were located more distally from the translation and transcriptional start site but were chosen because they have been implicated in atherosclerotic cardiac disease [4–6, 10, 19]. The third promoter SNP (–362 G/C) is located more proximally and lies in close proximity to a number of known transcription factor-binding regions. In particular, it is near the TRE region, known to be the site required to activate MCP-1 transcription in response to shear force on the vessel wall [20]. The remaining SNP in MCP-1 [903 C/T (rs4586)] is located in exon 2 of MCP-1; it has been haplotyped extensively and was included because of a previous association with giant cell arteritis and HIV [21, 22].

Bivariate analyses using standard χ^2 analysis were performed to discern any apparent associations between individual SNPs and carotid atherosclerosis. We then constructed haplotypes with the 4 chosen MCP-1 SNPs and conducted a bivariate analysis comparing individual haplotypes with carotid atherosclerosis. Multivariate analyses were also performed with adjustment for age, sex, risk factors and non-independence within families and carotid atherosclerosis. Finally, we performed a multivariate analysis to find interactions between any MCP-1 SNP of interest and the heterozygote SNP configuration of the CCR2 (rs1799864). No homozygote CCR2 V64I genotypes (rs1799864) were observed in the group without plaque, 1 was observed in our affected group with plaque and 5 in the population as a whole.

Table 1. Sample characteristics by plaque status (n = 325)

Characteristic	No plaque (n = 248)	Plaque (n = 77)	p
Age, years	45.6 ± 6.8	50.6 ± 5.8	<0.0001
SBP, mm Hg	133.6 ± 16.1	136.3 ± 16.2	0.20
DBP, mm Hg	86.3 ± 10.3	86.5 ± 9.4	0.84
Total cholesterol, mmol/l	5.4 ± 1.2	5.52 ± 1.1	0.41
Triglycerides, mmol/l	1.28 ± 0.76	1.59 ± 1.1	0.02
HDL cholesterol, mmol/l	1.41 ± 0.41	1.4 ± 0.41	0.91
LDL cholesterol, mmol/l	3.43 ± 1.1	3.44 ± 0.95	0.91
Glucose, mmol/l	5.44 ± 1.9	6.17 ± 2.4	0.02
BMI	31.4 ± 6.5	31.7 ± 7.4	0.78
Education, years	13.2 ± 2.5	12.6 ± 2.4	0.06
Male sex, %	28.63	40.26	0.05
Diabetic, %	8.06	22.08	0.0007
Current smoker, %	26.21	27.27	0.85
Hypertension, %	53.23	70.13	0.009
Obese, %	54.84	51.95	0.66

SBP = Systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; BMI = body mass index. To convert to mg/dl, divide total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol values by 0.0259, divide glucose values by 0.0555, and divide triglyceride values by 0.0113.

Table 2. Single nucleotide polymorphisms

Gene	SNP rs No.	SNP description	Frequency of minor allele	Frequency of major allele	HWE p
MCP1	rs1024611	–2578 A/G	0.167	0.833	0.672
MCP1	rs1024610	–2136 A/T	0.071	0.929	0.728
MCP1	rs2857656	–362 G/C	0.357	0.643	0.411
MCP1	rs4586	903 C/T	0.350	0.650	0.216
CCR2	rs1799864	Val64Ile A/G	0.127	0.873	0.961

HWE = Hardy-Weinberg equilibrium.

Genetic associations between SNPs and the presence of carotid artery plaque were assessed by comparing genotypic frequencies between subjects with and without plaque, using Fisher's exact test; the odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated. Multivariate logistic regression analyses were adjusted for age, sex, diabetes, current smoking status, systolic blood pressure, obesity, low-density lipoprotein cholesterol level and education level in our models. Non-independence within families was controlled for by using generalized estimating equations [23–25]. Best haplotype reconstruction frequencies were estimated using PHASE (version 2.1) [26, 27]. Haplotype frequencies between subjects with and without carotid artery plaque were screened by Fisher's exact test of 1 haplotype against all others; no association was found with any phenotype. Linkage disequilibrium was calculated between all pairs of markers using HAPLOVIEW [28]. Statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc., Cary, N.C., USA) and SUDAAN (version 9.0.3; Research Triangle Institute, Research Triangle Park, N.C., USA), unless otherwise stated.

Table 3. Associations of SNPs with carotid plaque

SNP	Model	Unadjusted p	Adjusted[1] p
rs2857656	additive	0.15	0.10
	dominant (CC vs. CG+GG)	0.10	0.05
	recessive (GG vs. CC+CG)	0.60	0.53
rs1024611	additive	0.94	–
	dominant (AA vs. AG+GG)	0.77	0.67
	recessive (GG vs. AA+AG)	0.78	0.85
rs1024610	additive	0.73	–
	recessive (TT vs. AA+AT)	0.92	0.88
rs4586	additive	0.98	0.92
	dominant (CC vs. CT+TT)	0.93	0.69
	recessive (TT vs. CC+CT)	0.84	0.82
rs1799864	additive	0.19	0.53
	recessive (GG vs. AA+AG)	0.081	0.36

[1] Adjusted for age, sex, diabetes, smoking, hypertension, obesity, low-density lipoprotein cholesterol and education.

Results

The final study population included 325 apparently healthy brothers (31%, n = 102) and sisters (69%, n = 223) of 185 probands with premature CAD (<60 years of age). No probands were included in the final analysis. The sample characteristics of this population by plaque status are described in table 1. The SNPs selected and their allele frequencies are listed in table 2. Seventy-seven subjects (24%) had evidence of carotid plaque (densities >1 mm). Using bivariable χ^2 analysis, we examined associations between individual SNPs and the presence of carotid artery plaque (table 3). One SNP, the MCP-1 –362 G/C (rs2857656), showed an association with carotid plaque in a recessive multivariate model. We calculated the degree of linkage disequilibrium (R^2) for all MCP-1 SNPs examined. The highest R^2 was 0.36 between rs1024611 and rs 2857656. The D prime value was high, but there was a low frequency of the minor alleles in the setting of

Table 4. Multivariate logistic regression estimating the risk of carotid plaque associated with the MCP-1 –362 CC genotype in interaction with the CCR2 64I SNP

Independent variables	OR	Lower 95% OR	Upper 95% OR	β ± SE	p
Age, years	1.13	1.08	1.19	0.123 ± 0.025	<0.0001
Male sex	1.68	0.94	3.00	0.521 ± 0.29	0.077
Diabetes	2.04	0.86	4.97	0.710 ± 0.43	0.104
Current smoker	1.24	0.61	2.53	0.216 ± 0.36	0.550
Hypertension	1.28	0.67	2.47	0.249 ± 0.33	0.454
Obese	0.68	0.36	1.31	–0.380 ± 0.33	0.250
LDL cholesterol, mmol/l	0.93	0.69	1.25	–0.00186 ± 0.0042	0.634
Education, years	0.91	0.80	1.05	–0.0899 ± 0.069	0.191
MCP-1 (rs2857656) and CCR2 (rs1799864) interaction (rs2857656 = CC and rs1799864 =AA or AG)	6.14	1.82	20.73	1.815 ± 0.696	0.0037

LDL = Low-density lipoprotein.

a low R^2 value, suggesting that the SNPs genotyped in this region are largely segregating independently.

In the multivariate adjusted analysis, a CC genotype at base –362 was associated with the presence of carotid artery plaque (OR 2.09, 95% CI 0.99–4.38; p = 0.05). The significance of the MCP-1 –362 G/C SNP (rs2857656) was independent of the other SNPs genotyped. Our analysis of the distal SNP MCP-1 –2578 A/G (rs1024611) and the CCR2 (rs1799864) in the multivariate model showed no relation between these SNPs and carotid plaque.

To address the issue of possible synergistic interaction between the –362G/C SNP in MCP-1 and the CCR2 (rs1799864), we performed multivariate logistic regression analysis, adjusting for the dependent variables listed above, but also including the MCP-1 –362 G/C SNP in a recessive model with the homozygote CC configuration and the CCR2 (rs1799864) A allele in homozygote or heterozygote configuration (i.e. AA + AG). Our analysis demonstrated a significant increase in the risk of carotid plaque in those subjects who were both homozygous (CC) for the MCP-1 –362 G/C SNP and homozygous or heterozygous for the CCR2 (rs1799864) A allele (AA or AG genotype) (OR 6.14, 95% CI 1.82–20.73) (see table 4).

Discussion

Recent investigations of the genetic risk factors that affect stroke have focused on the detection of stroke susceptibility factors. These susceptibility factors represent genetic traits that may be prevalent in populations which are beneficial under different circumstances at different times but may adversely affect individuals in old age or when exposed to stress from the environment. These genes can affect a wide array of biological systems and, under normal circumstances, may be beneficial. Other genetic stroke studies have focused on SNPs that affect genes in single molecules in biological systems associated with inflammation and endothelial activation. Examples of such molecules include matrix metalloproteinase type 2, cylcooxygenase 2, SERPINA-9 and the IL-2 receptor antagonist [29–32]. We chose to look at SNPs affecting a key inflammatory biological system: the chemokine MCP-1. It is part of inflammation and is a key mediator in a biological system that plays a definitive and early role in atherosclerosis formation. It is responsible for the attraction of macrophages to the area of damage caused by fatty streaks in the vessel wall. Numerous studies have demonstrated the significance of this chemokine in animal models of atherosclerosis. Apolipoprotein E knockout mice without the CCR2 receptor have an increased risk of atherosclerosis formation [12]. Apolipoprotein E mice with augmented lymphocytes with increased MCP-1 secretion also have an increased risk of atherosclerosis formation [11]. Human studies have associated biologically active SNPs in the MCP-1 promoter as well as increased MCP-1 levels with an increased risk of CAD [1–5, 17, 19, 33].

Studies involving the MCP-1/CCR2 biological system in human populations have previously focused on the –2578 A/G polymorphism (rs1024611) SNP in the MCP-1 promoter or on the CCR2 (rs1799864) receptor SNP. Both

of these SNPs have been independently associated with the development of CAD without epistatic or synergistic interactions [4–8, 10, 19, 33]. Ours is the first study to report any association with the proximal MCP-1 promoter SNP MCP-1 –362 G/C (rs2857656) and atherosclerosis. It is also the first study to demonstrate synergy between the SNP MCP-1 –362 G/C (rs2857656) in MCP-1 and an SNP in its receptor CCR2 (rs1799864) in the development of atherosclerosis of any type. The detection of synergy between the 2 sites separated on 2 different chromosomes operating on different parts of the MCP-1/CCR2 messenger system strengthens our study, supporting the hypothesis of the independent effect of the MCP-1 CCR2 messenger system on carotid atherosclerosis.

We chose the MCP-1 –362 G/C (rs2857656) SNP in consideration of unpublished data suggesting increased biological activity associated with the MCP-1 –362 G/C (rs2857656) SNP. Our data indicate that the MCP-1 –362 G/C (rs2857656) SNP creates a STAT binding site. Furthermore, we have created a luciferase construct, in which the –362 C SNP is associated with increased in vitro transcriptional activity. In addition, the SNP (–362 G/C) lies in close proximity to a number of known transcription factor-binding regions. In particular, it is near the TRE region, known to be the site required to activate MCP-1 transcription in response to shear force on the vessel wall [20]. The MCP-1 –362 G/C (rs2857656) SNP may increase transcription of MCP-1 and act in association with the CCR2 (rs1799864) SNP which increases the biological activity of the CCR2 receptor, to increase the biological activity of the MCP-1/CCR2 messenger system. The MCP-1/CCR2 system is known to affect the risk of atherosclerosis, and increased activity in the MCP-1/CCR2 system may increase the risk of atherosclerosis development.

Previous studies have noted an increased burden of disease from stroke and carotid atherosclerosis in African Americans [34–38]. There is an increase in the prevalence of the MCP-1 –362 SNP in African Americans while the CCR2 (rs1799864) SNP occurs with about equal frequency in both African Americans and Whites [22, 39]. It is possible the increased prevalence of the MCP-1 –362 SNP in the black population may contribute to the increased genetic susceptibility to carotid atherosclerosis observed in black populations [32, 40, 41]. Concordance of the MCP-1 SNP and the CCR2 SNP could be contributing to an increased risk of carotid atherosclerosis in African Americans. Confirmation of our findings in a different ethnic group, such as a white population, would support our assumptions. Examination of the effects of these SNPs on the prevalence of the carotid phenotype would allow us to make comparisons with black populations. In our sibling study population, the number of available Whites was not of sufficient size to allow for hypothesis testing. In addition, the lower frequency of the MCP-1 –362 G/C (rs2857656) SNP in white populations further reduced our power and limited the appropriateness of our available sample.

Our study requires repetition in larger populations. While the synergistic effect achieves acceptable significance with a p value of 0.0037, in this population of 325 individuals, the recessive model of the SNP MCP-1 –362 G/C (rs2857656) alone has a p value of 0.05 and demonstrates only borderline significance. The CCR2 (rs1799864) receptor SNP fails to achieve significance independently of the MCP-1 –362 G/C (rs2857656). This SNP has been strongly associated with CAD in larger populations, yet has never been associated with carotid atherosclerosis. It is necessary to repeat this study in a population with sufficient size to allow for the confirmation or refutation of an independent role of each of these SNPs on the risk of carotid atherosclerosis development as well as verifying their synergistic properties.

Conclusions

We detected synergy between MCP-1 SNP rs2857656 and an SNP in its receptor CCR2 (rs1799864). Our findings suggest that patients with both of these SNP variants may have an increased susceptibility to carotid atherosclerosis. African Americans have a greater frequency of the rs2857656 variant; therefore, they may also be at increased risk of carotid atherosclerosis. Additional studies in larger populations comparing Whites and African Americans are needed to confirm this possibility.

Acknowledgements

Special thanks to Tzipora Sofare, MA, for her expert editorial assistance. This work was supported by grants from the NIH/National Heart, Lung and Blood Institute (R18 HL58625 and U01 HL72518) and by a grant from the NIH/National Center for Research Resources (M01-RR000052) to the Johns Hopkins General Clinical Research Center.

References

1 Brenner D, Labreuche J, Touboul PJ, Schmidt-Petersen K, Poirier O, Perret C, Schonfelder J, Combadiere C, Lathrop M, Cambien F, Brand-Herrmann SM, Amarenco P: Cytokine polymorphisms associated with carotid intima-media thickness in stroke patients. Stroke 2006;37:1691–1696.

2 Cha SH, Lee JY, Kim HT, Ryu HJ, Han BG, Kim JW, Oh B, Kimm K, Shin HD, Park BL, Park S, Park HY, Jang Y: Association of CCR2 polymorphisms with the number of closed coronary artery vessels in coronary artery disease. Clin Chim Acta 2007;38:129–133.

3 Iwai N, Kajimoto K, Kokubo Y, Okayama A, Miyazaki S, Nonogi H, Goto Y, Tomoike H: Assessment of genetic effects of polymorphisms in the MCP-1 gene on serum MCP-1 levels and myocardial infarction in Japanese. Circ J 2006;70:805–809.

4 Jemaa R, Rojbani H, Kallel A, Ben Ali S, Feki M, Chabrak S, Elasmi M, Taieb SH, Sanhaji H, Souheil O, Mechmeche R, Kaabachi N: Association between the –2518G/A polymorphism in the monocyte chemoattractant protein-1 (MCP-1) gene and myocardial infarction in Tunisian patients. Clin Chim Acta 2008;390:122–125.

5 Kim MP, Wahl LM, Yanek LR, Becker DM, Becker LC: A monocyte chemoattractant protein-1 gene polymorphism is associated with occult ischemia in a high-risk asymptomatic population. Atherosclerosis 2007; 193:366–372.

6 McDermott DH, Yang Q, Kathiresan S, Cupples LA, Massaro JM, Keaney JF Jr, Larson MG, Vasan RS, Hirschhorn JN, O'Donnell CJ, Murphy PM, Benjamin EJ: CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. Circulation 2005;112:1113–1120.

7 Ortlepp JR, Vesper K, Mevissen V, Schmitz F, Janssens U, Franke A, Hanrath P, Weber C, Zerres K, Hoffmann R: Chemokine receptor (CCR2) genotype is associated with myocardial infarction and heart failure in patients under 65 years of age. J Mol Med 2003; 81:363–367.

8 Petrkova J, Cermakova Z, Drabek J, Lukl J, Petrek M: CC chemokine receptor (CCR)2 polymorphism in Czech patients with myocardial infarction. Immunol Lett 2003;88: 53–55.

9 Chen YL, Chang YJ, Jiang MJ: Monocyte chemotactic protein-1 gene and protein expression in atherogenesis of hypercholesterolemic rabbits. Atherosclerosis 1999;143: 115–123.

10 Rovin BH, Lu L, Saxena R: A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem Biophys Res Commun 1999;259:344–348.

11 Aiello RJ, Bourassa PA, Lindsey S, Weng W, Natoli E, Rollins BJ, Milos PM: Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 1999; 19:1518–1525.

12 Boring L, Gosling J, Cleary M, Charo IF: Decreased lesion formation in CCR2–/– mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 1998;394:894–897.

13 Dawson TC, Kuziel WA, Osahar TA, Maeda N: Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis 1999;143: 205–211.

14 Furukawa Y, Matsumori A, Ohashi N, Shioi T, Ono K, Harada A, Matsushima K, Sasayama S: Anti-monocyte chemoattractant protein-1/monocyte chemotactic and activating factor antibody inhibits neointimal hyperplasia in injured rat carotid arteries. Circ Res 1999;84:306–314.

15 Yu X, Dluz S, Graves DT, Zhang L, Antoniades HN, Hollander W, Prusty S, Valente AJ, Schwartz CJ, Sonenshein GE: Elevated expression of monocyte chemoattractant protein 1 by vascular smooth muscle cells in hypercholesterolemic primates. Proc Natl Acad Sci USA 1992;89:6953–6957.

16 Zhang J, Ding X: Identification and characterization of a novel tissue-specific transcriptional activating element in the 5′-flanking region of the CYP2A3 gene predominantly expressed in rat olfactory mucosa. J Biol Chem 1998;273:23454–23462.

17 Bjarnadottir K, Eiriksdottir G, Aspelund T, Gudnason V: Examination of genetic effects of polymorphisms in the MCP-1 and CCR2 genes on MI in the Icelandic population. Atherosclerosis 2006;188:341–346.

18 Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert panel on the identification, evaluation, and treatment of overweight in adults. Am J Clin Nutr 1998;68:899–917.

19 Cermakova Z, Petrkova J, Arakelyan A, Drabek J, Mrazek F, Lukl J, Petrek M: The MCP-1 -2518 (A to G) single nucleotide polymorphism is not associated with myocardial infarction in the Czech population. Int J Immunogenet 2005;32:315–318.

20 Ueda A, Ishigatsubo Y, Okubo T, Yoshimura T: Transcriptional regulation of the human monocyte chemoattractant protein-1 gene. Cooperation of two NF-kappaB sites and NF-kappaB/Rel subunit specificity. J Biol Chem 1997;272:31092–31099.

21 Amoli MM, Salway F, Zeggini E, Ollier WE, Gonzalez-Gay MA: MCP-1 gene haplotype association in biopsy proven giant cell arteritis. J Rheumatol 2005;32:507–510.

22 Modi WS, Goedert JJ, Strathdee S, Buchbinder S, Detels R, Donfield S, O'Brien SJ, Winkler C: MCP-1-MCP-3-eotaxin gene cluster influences HIV-1 transmission. Aids 2003;17:2357–2365.

23 Abecasis GR, Cardon LR, Cookson WO: A general test of association for quantitative traits in nuclear families. Am J Hum Genet 2000;66:279–292.

24 Horvath S, Xu X, Laird NM: The family based association test method: strategies for studying general genotype – phenotype associations. Eur J Hum Genet 2001;9:301–306.

25 Huber P: The behavior of maximum likelihood estimates under nonstandard conditions. Proc 5th Berkeley Symp Math Stat Probab, Berkeley, 1967.

26 Stephens M, Donnelly P: A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–1169.

27 Stephens M, Smith NJ, Donnelly P: A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989.

28 Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21: 263–265.

29 Fatar M, Stroick M, Griebe M, Hennerici M: Matrix metalloproteinases in cerebrovascular diseases. Cerebrovasc Dis 2005;20:141–151.

30 Furukado S, Sakaguchi M, Yamagami H, Yagita Y, Hoshi T, Abe Y, Hougaku H, Hori M, Sakoda S, Kitagawa K: Cyclo-oxygenase-2 –765G > C promoter variants are associated with lower carotid plaque echogenicity in Japanese. Cerebrovasc Dis 2009;27:91–98.

31 Morrison AC, Bare LA, Luke MM, Pankow JS, Mosley TH, Devlin JJ, Willerson JT, Boerwinkle E: Single nucleotide polymorphisms associated with coronary heart disease predict incident ischemic stroke in the atherosclerosis risk in communities study. Cerebrovasc Dis 2008;26:420–424.

32 Worrall BB, Azhar S, Nyquist PA, Ackerman RH, Hamm TL, DeGraba TJ: Interleukin-1 receptor antagonist gene polymorphisms in carotid atherosclerosis. Stroke 2003;34:790–793.

33 Tabara Y, Kohara K, Yamamoto Y, Igase M, Nakura J, Kondo I, Miki T: Polymorphism of the monocyte chemoattractant protein (MCP-1) gene is associated with the plasma level of MCP-1 but not with carotid intima-media thickness. Hypertens Res 2003;26: 677–683.

34 D'Agostino RB Jr, Burke G, O'Leary D, Rewers M, Selby J, Savage PJ, Saad MF, Bergman RN, Howard G, Wagenknecht L, Haffner SM: Ethnic differences in carotid wall thickness. The insulin resistance atherosclerosis study. Stroke 1996;27:1744–1749.
35 Kennedy BS, Kasl SV, Brass LM, Vaccarino V: Trends in hospitalized stroke for blacks and whites in the United States, 1980–1999. Neuroepidemiology 2002;21:131–141.
36 Sacco RL, Boden-Albala B, Gan R, Chen X, Kargman DE, Shea S, Paik MC, Hauser WA: Stroke incidence among white, black, and Hispanic residents of an urban community: the Northern Manhattan Stroke Study. Am J Epidemiol 1998;147:259–268.
37 Stewart JA, Dundas R, Howard RS, Rudd AG, Wolfe CD: Ethnic differences in incidence of stroke: prospective study with stroke register. BMJ 1999;318:967–971.
38 White H, Boden-Albala B, Wang C, Elkind MS, Rundek T, Wright CB, Sacco RL: Ischemic stroke subtype incidence among whites, blacks, and Hispanics: the Northern Manhattan Study. Circulation 2005;111:1327–1331.
39 Bielinski SJ, Pankow JS, Miller MB, Hopkins PN, Eckfeldt JH, Hixson J, Liu Y, Register T, Myers RH, Arnett DK: Circulating MCP-1 levels shows linkage to chemokine receptor gene cluster on chromosome 3: the NHLBI family heart study follow-up examination. Genes Immun 2007;8:684–690.
40 Rundek T, Elkind MS, Pittman J, Boden-Albala B, Martin S, Humphries SE, Juo SH, Sacco RL: Carotid intima-media thickness is associated with allelic variants of stromelysin-1, interleukin-6, and hepatic lipase genes: the Northern Manhattan Prospective Cohort Study. Stroke 2002;33:1420–1423.
41 Huang HY, Thuita L, Strickland P, Hoffman SC, Comstock GW, Helzlsouer KJ: Frequencies of single nucleotide polymorphisms in genes regulating inflammatory responses in a community-based population. BMC Genet 2007;8:7.