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

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Monocyte chemoattractant protein-1 (MCP-1) plays an important role in atherosclerosis. Recently, single nucleotide polymorphisms (SNPs) in the MCP-1 regulatory region have been identified, and an in vitro study demonstrated that the SNP at position -2518 of the MCP-1 gene affected transcription of the gene. The purpose of this study was to clarify the association of the plasma level of MCP-1 and the SNP of the MCP-1 gene with carotid atherosclerosis in community-based subjects. The study subjects consisted of 325 community residents, aged 50 years or older (mean age, 70.5 ± 9.4 years) and free from any cardiovascular complications. Carotid intima-media thickness (IMT) was measured in the right common carotid artery using ultrasonography. The plasma level of MCP-1 was measured by enzyme-linked immunosorbent assay (ELISA). The SNP of the MCP-1 gene was determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. The plasma level of MCP-1 was significantly associated with IMT (r = 0.12, p < 0.05) and carotid arterial dimension (r = 0.13, p < 0.05). There was a significant difference in plasma MCP-1 level between the genotypes (AA, 166 ± 36 ng/ml; GG + AG, 184 ± 56 ng/ml; p = 0.036). Analysis restricted to the subjects not receiving antihypertensive drugs or other medication further increased the statistical significance. However, carotid IMT and carotid arterial diameter were not significantly different among the MCP-1 genotypes. Stepwise regression analysis for plasma MCP-1 revealed that the MCP-1 genotype was an independent determinant of plasma MCP-1 level. These findings indicate that plasma MCP-1 is associated with carotid atherosclerosis. Although -2518 SNP is associated with the plasma level of MCP-1, it was not directly associated with carotid atherosclerosis. (Hypertens Res 2003; 26: 677–683)

Key Words: polymorphism, monocyte chemoattractant protein-1, carotid atherosclerosis

Introduction

There is accumulating evidence, both in vivo and in vitro, that monocyte chemoattractant protein-1 (MCP-1) plays an important role in atherosclerosis (1). MCP-1 shows potent chemotactic activity toward monocytes in response to immune, inflammatory, and mechanical stimuli, such as balloon injury (1–6). Expression of MCP-1 has been demonstrated in atherosclerotic lesions in animal models and humans (7–10). The plasma level of MCP-1 has also been shown to increase in patients with myocardial infarction (11, 12), unstable angina (13), venous thrombosis (14), and Kawasaki disease (15). It has also been demonstrated that
anti-MCP-1 neutralizing antibody prevents early inflammation and reduces subsequent coronary vascular medial thickening in N\textsuperscript{o}-nitro-L-arginine methyl ester (L-NAME)-administered rats (16). These findings suggest that continuous activation of MCP-1 is involved in the progression of atherosclerosis. Nevertheless, there have been only a few studies evaluating the relationship between MCP-1 and carotid atherosclerosis in humans (17).

Recently, single nucleotide polymorphisms (SNPs) in the MCP-1 regulatory region, i.e., substitutions of -2518 G/A, have been identified and shown to affect transcription of the gene (18). It has also recently been reported that the GG genotype of the -2518 MCP-1 gene was associated with susceptibility to coronary arterial disease (19). Aguilar et al. (20) demonstrated an association between the presence of G at position -2518 in the MCP-1 promoter region and the presence of cutaneous vasculitis among patients with systemic lupus erythematosus. However, there has been no study evaluating the association between -2518 SNP and MCP-1 level, which may account for the process of arterial remodeling.

In the present study, the plasma level of MCP-1 and the -2518 SNP of the MCP-1 gene were determined in 325 community residents, and their relationship with the carotid intima-media thickness (IMT) thickening and carotid arterial dilation was investigated to address the following unresolved issues: 1) whether the plasma level of MCP-1 is associated with the degree of carotid atherosclerosis; 2) whether the SNP at -2518 is associated with the plasma level of MCP-1; and 3) whether the SNP at -2518 is associated with carotid atherosclerosis.

**Methods**

**Subjects**

The Shimanami Health Promoting Program (J-SHIPP) was started in 1999 in the Shimanami district, located in the southern part of Japan (21). J-SHIPP is a longitudinal study evaluating factors relating to cardiovascular disease, dementia, and death. The present study is a part of J-SHIPP performed in a single community that participated in previous J-SHIPP studies. All residents aged over 50 years were invited to participate in the program, which consisted of an interview, anthropometric measurement, blood sampling, and carotid ultrasonography. About 50% of residents aged over 50 participated in the program. Among them, subjects aged over 50 and free from any history of symptoms of cardiovascular disease such as stroke, transient ischemic attack (TIA), myocardial infarction, angina, congestive heart failure, and peripheral vascular disease were enrolled in the study. Subjects with inflammatory disease or infections were excluded from the study. Informed consent for the procedure was obtained from each subject. All procedures were approved by the ethical committee of the Ehime University School of Medicine. Three hundred and twenty-five subjects completed the whole procedures.

**Evaluation of Carotid Artery**

The right carotid artery was evaluated with an SSD-900 (Aloka Co., Ltd., Tokyo, Japan) using a 7.5-MHz probe. After having the subject rest for at least 10 min in the supine position with the neck in slight hyperextension, we evaluated an optimal visualization of the right common carotid artery (CCA), carotid bulb, and extracranial internal and external carotid arteries. From anterior, lateral, and posterior approaches, IMT of the far wall was measured in the right common carotid artery 1 cm proximal to the bulb and averaged to obtain the mean IMT (22, 23). Two-dimensionally guided M-mode tracings of the right CCA at 1 cm proximal to the bulb were recorded in real time. Peak-systolic internal diameters (Ds) were obtained by continuous tracing of the intimal-luminal interface of the near and far walls of the CCA in 3 cycles and averaged. The axial resolution of the M-mode system was 0.1 mm.

For the analysis of carotid atherosclerosis, carotid IMT thickening was defined as IMT > 0.85 mm and carotid arterial dilatation was defined as Ds > 7 mm based on the mean + 0.5 SD values of studied subjects free from any known risk factors (21).

**Evaluation of Risk Factors**

Systolic and diastolic brachial blood pressure was measured twice at a 5-min interval in the supine position with an automatic oscillometric blood pressure recorder (HEM-705CP; OMRON Co., Ltd., Tokyo, Japan) during the carotid echo examination. The mean value of two measurements was obtained. The validity of the device and the reproducibility of its results have been established previously (24). Total cholesterol, high-density lipoprotein (HDL)-cholesterol, and glucose were determined by conventional methods.

**Determination of Plasma Level of MCP-1 and SNP of the MCP-1 Gene**

Blood was withdrawn into a tube containing EDTA. Plasma was quickly obtained by centrifugation, and kept at -80°C until assay. The plasma level of MCP-1 was measured in duplicate with an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA). The interassay variability was 6.3%, and the intra-assay variability was 6.2%.

Genomic DNA was extracted from peripheral blood samples using an extraction kit (QIAGEN GmbH; Qiagen, Hilden, Germany) (25). SNP of the regulatory region of the MCP-1 gene, located at position -2518 (G or A), was determined according to the published method (26). In brief, a 930 bp DNA segment including the polymorphic site was
amplified by polymerase chain reaction (PCR) using a set of oligonucleotide primers: 5'-CCGAGATGTTCCCAGCACA-3' and 5'-CTGCTTTGCTTGTGCCTCTT-3'. The PCR products were digested with one unit of Pvu II and separated by 3% agarose gel electrophoresis. If the polymorphism was 2518A, a unique Pvu II restriction site would be eliminated from this segment of the 5'-flanking region. The DNA segment from G/G homozygous individuals was digested into 708 and 222 bp fragments.

**Statistical Analysis**

All values are expressed as the means ± SD unless otherwise specified. Statistical comparisons among groups were performed by analysis of variance (ANOVA). Differences in prevalence among groups and Hardy-Weinberg’s equilibrium were analyzed by the χ² method. Stepwise regression analysis was performed to evaluate the association between plasma MCP-1 level, classical risk factors, and MCP-1 genotype. All analyses were performed using the software package JMP (SAS Institute, Cary, USA). Values of p < 0.05 were considered to indicate statistical significance.

**Results**

**Plasma MCP-1 and Carotid Atherosclerosis**

The clinical characteristics of all participants are summarized in Table 1. The mean plasma level of MCP-1 was 181 ± 54 ng/ml. The plasma MCP-1 level was significantly associated with age (Fig. 1). However, there were no significant associations between plasma MCP-1 and other classical risk factors, including sex, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, plasma glucose and smoking status, or between plasma MCP-1 and medication status (Table 2). The plasma level of MCP-1 was significantly associated with carotid IMT (r = 0.12, p < 0.05) and carotid arterial dimension (r = 0.13, p < 0.05). The relationship between carotid atherosclerosis and plasma MCP-1 is summarized in Fig. 2. Carotid IMT thickening defined as IMT > 0.85 mm and carotid arterial dilatation defined as Ds > 7 mm were associated with a significantly higher level of plasma MCP-1.

**Effect of MCP-1 Gene Polymorphism on Plasma Concentration of MCP-1 and Carotid Atherosclerosis**

The breakdown of the total 325 subjects by the -2518 SNP of the MCP-1 gene was as follows: 47 subjects had the AA genotype, 141 the GA genotype, and 137 the GG genotype. The genotype distribution of MCP-1 was in agreement with Hardy-Weinberg’s equilibrium (p = 0.88). The plasma levels of MCP-1 for each of the three genotypes are summarized in
Table 3. Plasma Monocyte Chemoattractant Protein-1 (MCP-1), Carotid Intima-Media Thickness (IMT), and Carotid Arterial Diameter (Ds) According to MCP-1 Genotype

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>GA</th>
<th>GG</th>
<th>GA + GG</th>
<th>AA vs. GA vs. GG</th>
<th>AA vs. GA + GG</th>
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<tbody>
<tr>
<td>Total population</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>n</td>
<td>47</td>
<td>141</td>
<td>137</td>
<td>278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>166 ± 36</td>
<td>184 ± 59</td>
<td>184 ± 54</td>
<td>184 ± 56</td>
<td>2.2</td>
<td>0.11</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.78 ± 0.13</td>
<td>0.80 ± 0.13</td>
<td>0.80 ± 0.14</td>
<td>0.80 ± 0.13</td>
<td>0.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Ds (mm)</td>
<td>6.7 ± 0.9</td>
<td>6.7 ± 0.9</td>
<td>6.5 ± 0.8</td>
<td>6.6 ± 0.9</td>
<td>3.8</td>
<td>0.024</td>
</tr>
<tr>
<td>Subjects without medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>34</td>
<td>98</td>
<td>100</td>
<td>198</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>163 ± 34</td>
<td>192 ± 59</td>
<td>182 ± 53</td>
<td>187 ± 56</td>
<td>3.5</td>
<td>0.031</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.77 ± 0.13</td>
<td>0.79 ± 0.13</td>
<td>0.78 ± 0.13</td>
<td>0.79 ± 0.13</td>
<td>0.4</td>
<td>0.65</td>
</tr>
<tr>
<td>Ds (mm)</td>
<td>6.5 ± 0.8</td>
<td>6.6 ± 0.9</td>
<td>6.4 ± 0.9</td>
<td>6.5 ± 0.9</td>
<td>1.9</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Analysis of variance (ANOVA) with additive as well as dominant models of MCP-1 genotype was performed.

Multiple Regression Analysis of Plasma MCP-1

To further investigate whether the MCP-1 genotype independently affects plasma MCP-1 level, stepwise regression analysis of the plasma MCP-1 level was performed with the following parameters: age, sex, systolic blood pressure, total cholesterol, HDL-cholesterol, plasma glucose, smoking status, medication status, and MCP-1 genotype (in the dominant model). The results revealed that the MCP-1 genotype, in addition to age, was an independent determinant of the plasma MCP-1 level (Table 4).

Discussion

MCP-1 has been shown to be a key factor initiating the inflammatory process of atherogenesis and sustaining the proliferative response to vessel injury (1, 2). Elevated levels of MCP-1 have been reported in patients with myocardial infarction as well as after myocardial reperfusion (7, 8, 27). These findings are consistent with the features of an immediate-early gene (1). Transcription of MCP-1 occurs at the early stage after vascular injury (28). Recently, Cipollone et al. (26) demonstrated that the MCP-1 level after percutaneous transluminal coronary angioplasty (PTCA) could predict restenosis. They observed significantly higher MCP-1 levels sustained for 180 days after PTCA in subjects who developed restenosis compared with those who did not. A similar finding has also been reported in patients after stent implantation (29). Schmidt and Stern (1) proposed a chronic stage of the effect of MCP-1, in which prolonged MCP-1 production could have autocrine/paracrine effects on monocytes and smooth muscle cells to promote the development of atherosclerosis. On the other hand, plasma MCP-1 can also increase in response to the secondary leukocyte accumulation as well as in response to vascular alterations (30). Vascular stresses including high blood pressure have been shown to lead to the infiltration and activation of leukocytes
in target tissues, where these cells contribute to the increment of plasma MCP-1 level (31).

In the present study, we observed a modest but significant positive association between plasma MCP-1 and carotid IMT in community residents free from any history or symptoms of cardiovascular disease. These findings indicate that circulating MCP-1 may play a role in the progression of chronic atherosclerotic lesions. We also observed that plasma MCP-1 was associated with carotid arterial dimension. These findings further support the hypothesis that MCP-1 may act in the chronic stage of arterial remodeling. However, the association between plasma MCP-1 level and carotid IMT observed in this study was weak compared with that in a previous report by Stork et al. (17). One possible explanation for this discrepancy is the difference of study design. In short, Stork and colleagues recruited study subjects who exhibited at least one site of > 1.0 mm IMT in the carotid artery system from their large cohort. Furthermore, they evaluated the mean maximum IMT in the carotid and femoral arteries, while our evaluation was carried out at 1 cm proximal to the carotid bulb. Because there are still no supportive epidemiological data available, this issue deserves further investigation. The influences of antioxidative factors and antiatherosclerotic reactivity are another possible explanation. Further examination involving these factors may reveal a stronger association between plasma MCP-1 level and carotid atherosclerosis.

In the present study, the IMT of the far wall of the right common carotid was evaluated from anterior, lateral, and posterior approaches, and the mean of measurements of three points was calculated as the IMT. Although carotid arterial morphology evaluated with the present method has been shown to be associated with the status of several pathophysiological factors (21, 32–38), measurements of carotid IMT at more points could reflect carotid atherosclerosis more accurately. This finding leaves open the possibility that MCP-1 gene polymorphism could affect more subtle atherosclerotic changes.

Several studies have reported an age-dependent augmentation of plasma MCP-1 level (39, 40). We also observed a significant positive association between age and the plasma level of MCP-1. Although the exact mechanism of the age-dependent augmentation of plasma MCP-1 is not understood, Gerli et al. (39) postulated an age-dependent shift in the cytokine network. Age-dependent augmentation of the plasma level of MCP-1 may indicate occult atherosclerotic lesions (40). However, the result of our stepwise regression analysis indicated that aging itself determines the plasma level of MCP-1 independent of carotid IMT. Other known classical risk factors had no effect on plasma MCP-1.

SNP in the promoter region of the MCP-1 gene has been identified at position - 2518 relative to the major transcriptional start site, and was shown to be related to the promoter activity (26). An in vitro study demonstrated that IL-1β-induced luciferase activity was significantly greater in cells transfected with constructs containing GA at the - 2518 position than in those containing AA at this position. Furthermore, it has also been observed that IL-1β-treated peripheral blood mononuclear cells from G carriers (GA + GG), and especially from GG carriers, produced more MCP-1 than cells from individuals homozygous for AA. Our finding that subjects with the AA genotype had lower plasma MCP-1 compared with G carriers is consistent with these in vitro results. However, a co-dominant effect is certainly more in favor of a phenotype-genotype association. A previous report demonstrated the co-dominant inheritance in the IL-1β-induced MCP-1 production, but not in the basal production level, in vitro (41). Since patients with known atherosclerotic disorders, who tend to produce high levels of plasma MCP-1 (7, 8, 27) and among whom there is a relatively high prevalence of G carriers (19), were not admitted in the present study, the plasma level of MCP-1 may mimic G-allele-dominant inheritance. To our knowledge, this is the first community-based report to examine the effect of the - 2518 SNP on the plasma level of MCP-1.

We also investigated carotid morphology in the three genotypes of MCP-1. However, there was no association between MCP-1 genotype and either carotid atherosclerosis or carotid arterial dilatation. These findings do not exclude the possibility that the SNP is related to atherosclerotic disorders. With the knowledge that plasma MCP-1 is increased after myocardial events, and an increased level after PTCA is associated with restenosis, we cannot exclude the possibility that the plasma MCP-1 level responds to events such as myocardial infarction that could be affected by MCP-1 genotype. Recently, a case-control study reported that the frequency of the -2518 GG genotype was significantly higher in patients with coronary arterial diseases, including myocardial infarction (19). Although the genotype distribution was significantly deviated from Hardy-Weinberg’s equilibrium, the findings indicate the possibility that the SNP of MCP-1 is associated with thromboembolic disorders. Furthermore, the positive association between plasma MCP-1 level and carotid IMT, examined using maximum IMT, was previously reported. Although only the mean IMT was evaluated in this study, the examination using maximum IMT, as well as plaque score, may clarify the involvement of the MCP-1 genotype on carotid atherosclerosis.

The prevalence of - 2518 SNP in our study was different

<table>
<thead>
<tr>
<th>Parameter</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>MCP-1 genotype</td>
<td>0.11</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Sex, BMI, systolic blood pressure, IMT, total-cholesterol, HDL-cholesterol, glucose, and medication status were not entered in the equation.

Table 4. Stepwise Regression Analysis for Plasma Monocyte Chemoattractant Protein-1 (MCP-1)
from that previously reported in Asians (26). However, this previously reported prevalence was calculated using only 16 subjects. The allele frequency in our population was 36% for the A allele and 64% for the G allele. The previously reported G allele frequency in Caucasians (n = 71) was 29%, suggesting a profound racial difference. A recent study from Hungary reported a G allele frequency of 23.9% in controls (n = 320) and 29.9% in patients with coronary artery disease (n = 318) (19). The higher prevalence of the G allele in the Japanese population and the relatively low prevalence of coronary arterial disease, together with the findings of the present study, indicate an ethnic difference in the role of the MCP-1 gene in the development of atherosclerosis.

There were several important limitations to this study. First, our subjects had no symptoms of cardiovascular disease. Thus we cannot exclude the possibility that the plasma MCP-1 levels, as well as the MCP-1 polymorphism, would show a stronger association with carotid atherosclerosis in subjects with known atherosclerotic disorders, particularly in light of a previous case-control study in which patients with the MCP-1 polymorphism were more susceptible to coronary artery disease (19). Second, the selection criteria of the study population were skewed to a relatively older population. We previously reported the age-related augmentation of genetic factors for the development of carotid atherosclerosis (32). In terms of the gene-age interaction, we considered that the present selection of subjects would provide specific predictive information for the development of carotid atherosclerosis. To ensure the present findings, however, further evaluation with a wide range of subjects will be needed.

In summary, the plasma level of MCP-1 showed a significant association with carotid IMT thickening and carotid arterial dilation. -2518 SNP in the promoter region of MCP-1 was significantly related to the plasma level of MCP-1 in this Japanese population. However, -2518 SNP did not directly correlate with carotid IMT or carotid diameter.

References


