Association of monocyte chemoattractant protein 1 gene polymorphism with susceptibility to nonfamilial idiopathic dilated cardiomyopathy

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KEYWORDS
Dilated cardiomyopathy; Monocyte chemoattractant protein 1 (MCP-1); Polymorphism; Risk factor

Summary
Background: The cytotoxic action of leukocytes is known to be a probable cause of the cardiac myocyte damage seen in idiopathic dilated cardiomyopathy (IDC). Monocyte chemoattractant protein 1 (MCP-1) contributes to enhanced leukocyte recruitment and activation resulting in chronic damage of cardiomyocytes. MCP-1 has been reported to be dynamically regulated in IDC and may contribute to the deterioration of left ventricular function. In addition, a polymorphism at −2518 (G/A) in the MCP-1 gene affects the level of MCP-1 expression in response to an inflammatory stimulus.

Methods and results: We genotyped the polymorphism at −2518 G/A in the MCP-1 gene in 73 Japanese patients with nonfamilial IDC and 349 healthy controls. The distribution of the MCP-1 genotypes in the IDC patients differed significantly from the controls (p = 0.016). In a dominant G allele model, there was a significant difference in the distribution of genotypes between the two groups (p < 0.01). The odds ratio for nonfamilial IDC associated with the GG vs. non-GG genotype was 10.4 (95% CI = 1.7–64.5) after adjustment for the confounding factors.

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Conclusions: These findings suggest that the G allele at −2518 in the MCP-1 gene may be a novel genetic marker of susceptibility to nonfamilial IDC.

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Introduction

Idiopathic dilated cardiomyopathy (IDC) is a primary heart muscle disease characterized by left ventricular dilatation and systolic dysfunction, with secondary diastolic dysfunction. Although inherited gene defects account for 25–30% of IDC cases [1], most cases of IDC are sporadic and consistent with multifactorial genetic traits (nonfamilial IDC) [2,3]. As for the pathogenesis of nonfamilial IDC, persistent viral infection [3] and immunological abnormalities including autoimmunity [4] have been postulated. Because genetic factors influence the immune response to autoantigens as well as to foreign antigens, polymorphism in the genes involved in inflammation has been investigated in IDC, especially focusing on HLA [5]. Recent experimental and clinical data suggest a causal relationship between myocarditis and IDC, indicating that a chronic inflammatory process may be responsible for the development of IDC [3,6]. The cytotoxic action of leukocytes appears to be the most probable cause of cardiomyocyte damage seen in chronic myocarditis and IDC [6]. Monocyte chemoattractant protein 1 (MCP-1) contributes to enhanced leukocyte recruitment and activation resulting in chronic damage of cardiomyocytes [6]. Recently, MCP-1 has been reported to be dynamically regulated in IDC and may contribute to the deterioration in left ventricular function [6]. In addition, a polymorphism at −2518 (G or A) in the MCP-1 gene has been reported to affect the level of MCP-1 expression in response to an inflammatory stimulus [7]. However, there have been no studies evaluating the association between this polymorphism and nonfamilial IDC.

In the present study, we investigated MCP-1 polymorphism to determine whether this polymorphism was associated with susceptibility to nonfamilial IDC.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood samples with an extraction kit (Qiagen, Hilden, Germany). For detection of the −2518 G/A polymorphism of the MCP-1 gene, genomic DNA was amplified with primers (the forward primer 5′-CCG AGA TGT TCC CAG CAC AG-3′ and the reverse primer 5′-CTG CTT TGC TTG TGC CTC TT-3′) for the distal regulatory region of the MCP-1 gene as previously described [7]. The −2518 G/A polymorphism affected PvuII digestion of the MCP-1 distal regulatory region. The G allele gave two fragments obtained from each subject. In this study, 73 genetically unrelated Japanese patients with nonfamilial IDC were enrolled (58 males and 15 females, from 12 to 74 years, mean age at initial clinical evaluation 48.6 ± 14.4 years, mean body mass index 23.4 ± 4.2). The control group consisted of 349 healthy individuals free from any history or symptoms of cardiovascular disease (114 males and 235 females; from 52 to 97 years; mean age, 71.4 ± 9.4 years; mean body mass index, 23.1 ± 3.2). All healthy individuals were recruited from the Shimanami Health Promoting Program (J-SHIPP) [8]. All patients with IDC were diagnosed at Ehime University Hospital by examinations with 12-lead electrocardiography, transthoracic echocardiography, and cardiac catheterization. These patients also met the definition and classification proposed by the 1995 World Health Organization/International Society and the Federation of Cardiology Task Force [9]. The exclusion criteria were as follows: (a) history of myocardial infarction; (b) history of alcohol abuse; (c) evidence of coronary heart disease or severe valvular heart disease by cardiac catheterization; and (d) active myocarditis. In addition, patients having an apparent family history (at least one IDC patient in the first-degree family relatives) were excluded from the study, because the IDC phenotype in these patients was attributable to a monogenic defect in the known disease-causing genes. Any previous cardiac evaluations in the patients enrolled with IDC were reviewed. The initial clinical status of the patients was determined on the basis of medical records.

Methods

Study subjects

The study protocols were approved by the Ethical Committee of Ehime University School of Medicine, and written and informed consent was
Table 1 Characteristics of patients with IDC at initial clinical evaluation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at initial clinical evaluation (years)</td>
<td>48.6 (12—74)</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>58/15</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.4 (15—45)</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>2.4 (1—4)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81 (50—128)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115 (80—150)</td>
</tr>
<tr>
<td>LVDD (mm)</td>
<td>63 (50—102)</td>
</tr>
<tr>
<td>LVDS (mm)</td>
<td>51 (34—93)</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>19 (5—32)</td>
</tr>
</tbody>
</table>

Mean value (minimum value – maximum value). IDC, idiopathic dilated cardiomyopathy. NYHA, New York Heart Association. LVDD, left ventricular dimension at end-diastole. LVDS, left ventricular dimension at end-systole.

(708 bp and 222 bp), while the A allele gave a single 930-bp fragment [7]. Each PCR product was electrophoresed in 3% agarose gel and DNA was visualized directly with ethidium bromide staining.

Statistical methods

All statistical analyses were performed on a personal computer with SPSS Version 10.0J for Windows (SPSS, Inc, Chicago, IL, USA). Differences in the prevalence among groups and Hardy—Weinberg’s equilibrium were analyzed by the chi-square method. To analyze differences between G allele carriers and non-carriers, the AG and GG genotypes were pooled into one group. Association between polymorphism and case/control status was tested by logistic regression analysis controlling for age, gender, and body mass index. Odds ratios were estimated with 95% confidence intervals as measures of risk. A p-value of less than 0.05 was considered statistically significant.

Table 2 Distribution of genotypes and alleles for polymorphism (−2518 G/A) in the MCP-1 gene.

<table>
<thead>
<tr>
<th>MCP-1 gene (−2518 G/A)</th>
<th>IDC (n = 73)</th>
<th>Healthy Japanese (n = 349)</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype, number of subjects (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>2 (3)</td>
<td>52 (15)</td>
<td>0.016</td>
</tr>
<tr>
<td>A/G</td>
<td>33 (45)</td>
<td>149 (43)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>38 (52)</td>
<td>148 (42)</td>
<td></td>
</tr>
<tr>
<td>G dominant model, number of subjects (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>2 (3)</td>
<td>52 (15)</td>
<td>0.005</td>
</tr>
<tr>
<td>A/G + G/G</td>
<td>71 (97)</td>
<td>297 (85)</td>
<td></td>
</tr>
<tr>
<td>Allele, number of alleles (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A allele</td>
<td>37 (25)</td>
<td>253 (36)</td>
<td>0.012</td>
</tr>
<tr>
<td>G allele</td>
<td>109 (75)</td>
<td>445 (64)</td>
<td></td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemoattractant protein-1; IDC, idiopathic dilated cardiomyopathy.

Results

The initial clinical characteristics of the patients are summarized in Table 1. As shown in Table 2, the genotype distributions in the patients with nonfamilial IDC and the healthy control population were in Hardy—Weinberg’s equilibrium. The distribution of the MCP-1 genotypes was significantly different between the patients and the healthy subjects (p = 0.016). There was a significant difference in the frequency of alleles between the two groups (p = 0.012). In a dominant G allele model, there was a significant difference between the two groups (p < 0.01). Table 3 shows the odds ratios for nonfamilial IDC determined by logistic regression analysis. With subjects homozygotic for the A allele considered as the reference category, subjects heterozygotic for this polymorphism had increased risk for nonfamilial IDC. In addition, subjects homozygotic for the G allele had a risk of nonfamilial IDC that was 11 times greater than subjects homozygotic for the A allele. The risk was independent of other variables, because it was even higher when the estimate was adjusted for all of the other variables (age, gender, and body mass index). Patients with two G alleles had greater risk for nonfamilial IDC than patients with no or one G allele, which showed dose—response effect of this polymorphism. However, we could not find a relationship between severity of the disease and this polymorphism.

Discussion

This study is the first report of an important association between the MCP-1 gene polymorphism and nonfamilial IDC.
The −2518 A to G polymorphism is known to affect the transcriptional activity of this region and correlates with individual differences in monocyte MCP-1 production [7]. Monocytes from individuals carrying the G allele produced more MCP-1 after treatment with IL-1 than monocytes from A/A homozygous individuals [7]. Individuals with the G allele may be more responsive to several inflammatory stimuli than individuals without it. The high prevalence of the G allele in our IDC patients is compatible with a chronic inflammatory process in the pathogenesis of nonfamilial IDC.

It has been reported that circulating levels of MCP-1 were increased in patients with acute myocarditis [10], acute myocardial infarction [11], and congestive heart failure [12], and were also correlated with the severity of the disease. Activation and migration of leukocytes to areas of inflammation are important factors in these immunologic responses. Seino et al. showed the presence of MCP-1 messenger RNA in endomyocardial biopsy tissue obtained from patients with IDC and suggested a role for this chemokine in the regulation of inflammatory cell infiltration into the myocardium [13]. In addition, Lehmann et al. reported that IDC patients with severe left ventricular dysfunction showed a 2.35-fold higher MCP-1 messenger RNA expression in endomyocardial biopsy tissue when compared to IDC patients with milder dysfunction [6]. Furthermore, there was a consistent trend toward a higher infiltration of inflammatory cells in IDC patients with a lower ejection fraction [6]. According to these reports, MCP-1 may play an important role in the pathogenesis and the severity of IDC. The −2518 polymorphism was associated with the plasma level of MCP-1 in our control subjects (J-SHIPP) [8]. However, we could not examine the circulating MCP-1 levels in the patients with IDC, which was one of our study limitations. Although elevated circulating levels of MCP-1 are not specific for IDC, we believe that our findings support the notion that immunologic and inflammatory processes may be important features of IDC.

We found frequencies of the G and A alleles of 64% and 36%, respectively in healthy Japanese subjects. A recent study in Japanese subjects also demonstrated that the ratio of G to A allele was 65% to 35% [14], indicating that the G allele is indeed the predominant type in the Asian population. However, these data are discrepant with previously reported results for Caucasians and African Americans, where the G allele frequency was 29% and 22%, respectively [7]. Many studies, although controversial, have suggested that the G/G genotype of the MCP-1 gene is associated with the pathogenesis of Kawasaki disease [14], asthma severity [15], coronary artery disease [16], nephritis in systemic lupus erythematosus [17], and carpal-tunnel syndrome in hemodialysis patients [18].

The number of patients in our study was small, and our results should be compared with those of larger studies in various countries. Further molecular and biological studies are needed to clarify the relation between the MCP-1 polymorphism and IDC. In addition, it may be important to determine whether there are racial differences in inflammatory responses and racial variations in the frequency of this polymorphism.

In conclusion, this study strongly suggests that the G allele at −2518 in the MCP-1 gene may be a novel genetic marker of susceptibility to IDC in the Japanese population.

### References


