Association of Peripheral Mononuclear Cells Containing Chlamydia Pneumoniae DNA with Acute Coronary Syndrome and Stable Coronary Artery Disease in Japanese Subjects

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To clarify the association of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells (PBMCs) with acute coronary syndrome (ACS) and stable coronary artery disease (CAD) in Japanese adults, touchdown-nested polymerase chain reaction was used to detect the presence of C. pneumoniae DNA. The prevalence of C. pneumoniae DNA in PBMCs was similar in a comparison of 88 patients (52.3%) with ACS, 164 patients (50.0%) with stable CAD, and 88 control subjects (50.0%). Temporal changes in the prevalence of C. pneumoniae DNA in PBMCs were also assessed every 3 months during a 1-year period (n = 59). The prevalence was significantly higher in the first 3-month period (January through March) than in any of the other 3-month periods. In conclusion, the prevalence of C. pneumoniae DNA in PBMCs in patients with ACS or stable CAD was comparable to that in control populations. Furthermore, the presence of circulating C. pneumoniae was strongly associated with seasonal variability.

Chlamydia pneumoniae is an intracellular pathogen that is a common cause of respiratory tract infections worldwide. More than 60% of adults are infected with C. pneumoniae during their lifetime, and reinfections are common [1]. Since the report by Saikku et al. [2] describing elevated titers of IgG and IgA antibodies against C. pneumoniae in patients with recent myocardial infarction and chronic coronary artery disease (CAD), many studies have been performed to clarify the relationship between C. pneumoniae and atherosclerotic disease. C. pneumoniae has been detected in human atherosclerotic lesions by PCR [3, 4], immunohistochemistry [3, 4], electron microscopy [3], and cell culture [5–7], albeit in varying rates. Furthermore, viable pathogens have been recovered from atherosclerotic lesions in coronary arteries [5–7] and carotid arteries [6]. Several in vitro studies [8–10] have demonstrated that C. pneumoniae infects endothelial cells, smooth muscle cells, and macrophages and reproduces within them. Laitinen et al. [11] demonstrated that intranasal infection with C. pneumoniae causes inflammatory atherosclerosis-like changes in the aorta of the rabbits. In a mouse model, Moazed et al. [12] demonstrated that C. pneumoniae infection disseminates systemically through the bloodstream after infection of alveolar macrophages. Several investigators have proposed that C. pneumoniae continues to live within macrophages and migrates along with them into the arterial wall. Other investigators have examined the relationship between C. pneumoniae DNA in PBMCs and CAD [13–18], carotid atherosclerosis [19, 20], aortic aneurysms [21, 22], and vascular surgery [23].

Despite these data, it is still unclear whether the presence of C. pneumoniae DNA in PBMCs can be a useful indicator of CAD. Therefore, the goal of this study was to clarify the relationship between C. pneumoniae DNA in PBMCs and stable CAD or acute coronary syndrome...
(ACS) in Japanese adults. In addition, we examined the presence or absence of seasonal variability in the prevalence of *C. pneumoniae* DNA in PBMCs.

**MATERIALS AND METHODS**

**Subjects in the first portion of the study.** The prevalence of *C. pneumoniae* DNA in PBMCs was compared among 88 patients with ACS (59 with acute myocardial infarction and 29 with unstable angina pectoris), 164 with stable CAD, and 88 control subjects. Eligible patients with ACS or stable CAD who were admitted to our university or to 2 affiliated hospitals during the daytime between December 2001 and March 2002 and who met the following inclusion criteria were consecutively enrolled. Control subjects in the following patient groups were also enrolled in this study: outpatients at our university hospital with no evidence of CAD (e.g., no history of chest pain, no electrocardiographic abnormalities at rest, and a negative exercise stress test result; *n* = 38), or patients who underwent diagnostic coronary arteriography because of chest pain at our university or at 2 affiliated hospitals during the study period, without any subsequent evidence of significant coronary artery stenosis or coronary spasm (*n* = 50). Inclusion criteria for all study populations were as follows: age, >40 years; no history of upper or lower respiratory tract infections or use of antibiotics within 4 weeks of the blood sampling; and no history of chronic respiratory tract disease. A diagnosis of acute myocardial infarction was made on the basis of the following criteria: typical chest pain lasting for >20 min, characteristic changes noted on the electrocardiograph, and a creatine kinase level of more than twice the upper limit of normal. Unstable angina pectoris was defined according to the Braunwald classification [24]. All patients with ACS or with stable CAD underwent coronary arteriography. Patients with stable CAD had >50% stenosis in ≥1 coronary artery or had undergone prior percutaneous coronary intervention in ≥1 vessel.

**Subjects in the second portion of the study.** Blood samples were obtained from subjects every 3 months to assess temporal changes in the prevalence of *C. pneumoniae* DNA in PBMCs. The subjects consisted of 60 persons who were recruited from the first portion of the study from January 2002 through March 2002 and who were able to see a physician (A.T.) at least once during each 3-month period for 1 year. The subject selection was completed before the results of the first portion of the study were obtained. One patient dropped out before the completion of the study. The other 59 subjects (27 patients with CAD and 32 control subjects) completed the study. All study protocols were in agreement with the guidelines of the ethics committee at our institution, and informed consent was obtained from each patient before the study.

**PCR.** PCR was performed according to the methodology described by Dowell et al. [25]. Whole venous blood samples (6 mL) from each patient were collected in EDTA-treated tubes. Blood samples were obtained at hospital admission from patients with ACS, patients with stable CAD, and control subjects without abnormal findings on coronary arteriographs. Blood samples for touchdown-nested PCR were processed in accordance with the method described by Condos et al. [26]. Blood samples were carefully layered over Separa-L (Muto Pure Chemicals) at a ratio of blood to Separa-L of 3:4. The buffy coat was obtained by centrifugation (700 g for 30 min, brake off) and then washed with phosphate-buffered saline (700 g for 10 min, brake on) in a total volume of 10 mL. Finally, the resulting PBMCs were eluted to 200 μL of PBS and transferred to a new tube for DNA extraction.

DNA was extracted from PBMCs using a DNA minikit (QIImmP; Qiagen) in accordance with the manufacturer’s instructions. DNA was eluted to a final volume of 200 μL and stored at −20°C. Before we conducted PCR, the concentration of the DNA was analyzed by photometer (optical density, 260 nm). The average of 3 determinations was used.

The touchdown-nested PCR was performed as described elsewhere by Tong and Sillis [27] and Mahony et al. [28]. The gene coding for the major outer membrane protein (*ompA*) was amplified. The outer pair of primers (CP-1, 5′-TTACAAGCCTTGCCGTAGG-3′; CP-2, 5′-GGATGCCAAATGTTTACAGG-3′) were common to both the *ompA* gene of *C. pneumoniae* and *Chlamydia psittaci*, and the inner pair of primers (CPC, 5′-TTATTAATTGATGTTACAATA-3′; CPD, 5′-ATCTACGGCCAGTTAGTATAGT-3′) were specific to a variable domain of the *ompA* gene of *C. pneumoniae*. The outer primers amplified a 333-bp fragment, whereas the inner primers amplified a 207-bp fragment. The amplification reaction was performed in a volume of 100 μL containing 0.8 μg of extracted DNA, 10 mmol/L Tris HCl (pH, 8.3), 50 mmol/L KCl, and a 200-μmol/L concentration of 4 deoxynucleoside triphosphates. The ratio of a volume of template DNA to volume of reaction mix was determined as indicated by Mahony et al. [28]. Touchdown-nested PCR conditions were as follows: the first round of amplification employed 1.5 mmol/L MgCl₂, 0.4 μmol/L primers, and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer) and involved 20 cycles of 1 min at 94°C, 1 min at 65°C decreased by 0.5°C per cycle, and 1 min at 72°C, plus an additional 20 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The PCR products amplified by outer primers (CP-1–CP-2) were filtered by a microcon-PCR (Millipore), and a volume of 10 μL was added to a new 100-μL PCR mixture for a second amplification. The second round of amplification employed 3 mmol/L MgCl₂, 1 μmol/L primers, and 2.5 U of AmpliTaq Gold DNA polymerase and involved 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. The amplification products were stored at −20°C until the second portion of the study.
Table 1. Characteristics of subjects in a study of the prevalence of Chlamydia pneumoniae DNA in PBMCs.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with ACS (n = 88)</th>
<th>Patients with stable CAD (n = 164)</th>
<th>Control subjects (n = 88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>67 ± 12</td>
<td>67 ± 10</td>
<td>66 ± 12</td>
</tr>
<tr>
<td>BMI, mean</td>
<td>24.1</td>
<td>24.2</td>
<td>23.4</td>
</tr>
<tr>
<td>Male sex</td>
<td>62 (70.1)</td>
<td>127 (77.4)</td>
<td>63 (71.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>61 (69.3)</td>
<td>126 (76.8)</td>
<td>58 (65.9)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>54 (61.4)</td>
<td>117 (71.3)</td>
<td>51 (58.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>26 (29.5)</td>
<td>71 (43.3)</td>
<td>13 (14.8)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>20 (22.7)</td>
<td>65 (39.6)</td>
<td>9 (10.2)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>40 (45.5)</td>
<td>54 (32.9)</td>
<td>27 (30.7)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease.

Diabetes mellitus 26 (29.5) 71 (43.3) 13 (14.8)
Hyperlipidemia 54 (61.4) 117 (71.3) 51 (58.0)
Hypertension 61 (69.3) 126 (76.8) 58 (65.9)
Male sex 62 (70.1) 127 (77.4) 63 (71.6)
Age, mean years ± SD 67 ± 12 67 ± 10 66 ± 12
BMI, mean 24.1 24.2 23.4
Ex-smoker 20 (22.7) 65 (39.6) 9 (10.2)
Current smoker 40 (45.5) 54 (32.9) 27 (30.7)

were visualized in 2% agarose with ethidium bromide staining by standard techniques. Purified C. pneumoniae DNA from strain YK-41 was used as a positive control. The lower detection limit of the PCR was 0.1 inclusion-forming units. PCR was performed with primers for a β-globin gene sequence to confirm that the total DNA quantity of each sample was similar and that no false-negative results were generated [13].

To avoid the risk of contamination, all reactions were performed under stringent conditions. The DNA extraction, first PCR amplification, second PCR amplification, and electrophoresis were performed in separate rooms. Aerosol barrier pipette tips, dedicated laboratory coats, and gloves were also used. Four negative controls, consisting of sterile distilled water in place of the clinical specimen, were run along with every 5 samples for all reactions. Two sets of negative controls were incorporated at the DNA extraction stage, and additional negative controls were added during the first and second PCR steps. In addition, we examined the reproducibility and validity of our PCR method by repeated PCR tests and sequencing of positive PCR products, respectively.

Data analysis. Data are expressed as mean ± SD or as a percentage. Categorical data were analyzed by Fisher’s exact test or by the χ² test with Yates’ correction. Continuous variables were analyzed by the unpaired t test or by 1-way analysis of variance. A P value of <.05 was considered to be statistically significant.

RESULTS

The prevalence of C. pneumoniae DNA in PBMCs. Table 1 shows the characteristics of the study populations included in the first portion of the study. The frequency of persons with diabetes mellitus and of ex-smokers was significantly higher among patients with ACS and those with stable CAD than among control subjects. There was no significant difference in the prevalence of positive C. pneumoniae DNA in PBMCs when comparing the 3 groups (patients with ACS, 46 [52.3%] of 88; patients with stable CAD, 82 [50.0%] of 164; and control subjects, 44 [50.0%] of 88).

Temporal changes in the prevalence of C. pneumoniae DNA in PBMCs. Table 2 shows the characteristics of the study populations included in the second portion of the study. There was a significantly higher proportion of ex-smokers among patients with CAD patients than among control subjects (44.4% vs. 12.5%; P < .01). As is illustrated in figure 1, there was no significant difference in the prevalence of C. pneumoniae–positive DNA in PBMCs when comparing patients with CAD with control subjects in the different time periods (January through March, 16 [59.3%] of 27 vs. 19 [59.4%] of 32; April through March, 19 [59.4%] of 32 vs. 20 [59.4%] of 34).

Table 2. Characteristics of subjects in a study of temporal changes in the prevalence of Chlamydia pneumoniae DNA in PBMCs.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with CAD (n = 27)</th>
<th>Control subjects (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>69 ± 8</td>
<td>66 ± 13</td>
</tr>
<tr>
<td>BMI, mean</td>
<td>24.0</td>
<td>23.3</td>
</tr>
<tr>
<td>Male sex</td>
<td>21 (77.8)</td>
<td>24 (75.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (77.8)</td>
<td>24 (75.0)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>23 (85.2)</td>
<td>22 (68.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 (29.6)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>12 (44.4)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>7 (25.9)</td>
<td>5 (15.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease.

![Figure 1](https://academic.oup.com/cid/article-abstract/39/3/366/352631/figure1)

Figure 1. Temporal changes in the prevalence of Chlamydia pneumoniae DNA in PBMCs in 27 patients with coronary artery disease (CAD) and in 32 control subjects. *P < .05 versus January through March in patients with CAD; **P < .05 versus January through March in control subjects.
was the first to suggest a relationship between *C. pneumoniae* DNA in PBMCs and CAD. However, Boman et al. [13] demonstrated no significant difference in the prevalence of *C. pneumoniae* DNA in PBMCs when comparing patients with CAD and middle-aged blood donors (59.4% vs. 46.1%). In contrast, Wong et al. [14] reported that the prevalence of *C. pneumoniae* DNA in PBMCs was significantly higher in men with angiographically confirmed CAD than in those with normal coronaries (8.8% vs. 2.9%) and concluded that the presence of circulating *C. pneumoniae* DNA was a stronger predictor of CAD than were any of the known risk factors, such as smoking, hypercholesterolemia, hypertension, diabetes mellitus, and family history of CAD (OR, 3.2; 95% CI, 1.2–8.9). Sessa et al. [16] recently reported that the prevalence of *C. pneumoniae* DNA in PBMCs was significantly higher in patients with ACS than in healthy subjects (25.8% vs. 4.8%).

In the present study, there were no significant differences in the prevalence of *C. pneumoniae* DNA in PBMCs when comparing patients with ACS, those with stable CAD, and control subjects. There are several possible explanations for the discrepancies among the published studies, including the present study. First, each study involved unique patient populations that may have differences in the relationship between *C. pneumoniae* DNA in PBMCs and CAD. Second, the present study demonstrated seasonal variability in the prevalence of *C. pneumoniae* DNA in PBMCs. Thus, potential intergroup variability in the time that blood samples were obtained may lead to intergroup differences in the prevalence of *C. pneumoniae* DNA in PBMCs. In the present study, there were no significant differences in the prevalence of *C. pneumoniae* DNA in PBMCs in any 3-month period when comparing 27 patients with CAD and 32 control subjects. Thus, circulating *C. pneumoniae* DNA is unlikely to be a useful indicator of CAD or ACS.

**Seasonal variability in the prevalence of *C. pneumoniae* DNA in PBMCs.** Table 5 shows the seasonal change in the prevalence of *C. pneumoniae* DNA in PBMCs. Intermonth differences in the prevalence of *C. pneumoniae* DNA in PBMCs have been suggested by previous studies [17, 29–31]. Smieja et al. [17, 29] found a higher prevalence of *C. pneumoniae* DNA in winter and spring than in other seasons. Rassu et al. [30] demonstrated seasonal variability in the prevalence of *C. pneumoniae* DNA in PBMCs.

### Table 3. Serial PCR test results for 27 patients with coronary artery disease (CAD) and 32 control subjects.

<table>
<thead>
<tr>
<th>No. of positive PCR test results</th>
<th>All subjects (n = 59)</th>
<th>Patients with CAD (n = 27)</th>
<th>Control subjects (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8 (13.6)</td>
<td>4 (14.8)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>1</td>
<td>25 (42.4)</td>
<td>13 (48.1)</td>
<td>12 (37.5)</td>
</tr>
<tr>
<td>2</td>
<td>20 (33.9)</td>
<td>7 (25.9)</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td>3</td>
<td>6 (10.2)</td>
<td>3 (11.1)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 4. Studies describing the relationship between Chlamydia pneumoniae DNA in PBMCs and coronary artery disease (CAD).

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Subject group</th>
<th>Case patients</th>
<th>No. of subjects</th>
<th>Prevalence, %</th>
<th>Control subjects</th>
<th>No. of subjects</th>
<th>Prevalence, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boman et al. [13]</td>
<td>1998</td>
<td>Sweden</td>
<td>Patients with CAD</td>
<td>101</td>
<td>59.4</td>
<td></td>
<td>Blood donors</td>
<td>52</td>
<td>46.1</td>
<td>NS</td>
</tr>
<tr>
<td>Wong et al. [14]</td>
<td>1999</td>
<td>UK</td>
<td>Patients with CAD</td>
<td>913</td>
<td>8.7</td>
<td></td>
<td>Patients with normal angiogram findings</td>
<td>292</td>
<td>7.2</td>
<td>NS*</td>
</tr>
<tr>
<td>Sessa et al. [16]</td>
<td>2001</td>
<td>Italy</td>
<td>Patients with AMI and UAP</td>
<td>93</td>
<td>25.8</td>
<td></td>
<td>Healthy control subjects</td>
<td>42</td>
<td>4.8</td>
<td>.008</td>
</tr>
<tr>
<td>Smieja et al. [17]</td>
<td>2001</td>
<td>Canada</td>
<td>Patients with CAD</td>
<td>187</td>
<td>11.8</td>
<td></td>
<td>Patients with normal angiogram findings</td>
<td>21</td>
<td>9.5</td>
<td>NS</td>
</tr>
<tr>
<td>Present study</td>
<td></td>
<td>Japan</td>
<td>Patients with ACS</td>
<td>88</td>
<td>52.3</td>
<td></td>
<td>Patients with normal angiogram findings or no evidence of CAD</td>
<td>88</td>
<td>50.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** ACS, acute coronary syndrome; AMI, acute myocardial infarction; UAP, unstable angina; UK, United Kingdom; US, United States.

* The prevalence of Chlamydia pneumoniae DNA in PBMCs was significantly higher in men with angiographically confirmed CAD than in those with normal coronaries (8.8% [59 of 669]) vs. 2.9% ([4 of 135]), P < 0.05.

found a higher prevalence of *C. pneumoniae* DNA in PBMCs in February than in October (57.6% vs. 37.9%). Furthermore, Haranaga et al. [31] found a lower prevalence of *C. pneumoniae* DNA in PBMCs in October and November than in August and September (0.0% vs. 21.0%) in 237 healthy blood donors. In the present study, the prevalence of *C. pneumoniae*-positive DNA in PBMCs was significantly higher during the first 3-month period (January through March) than during other 3-month periods. Furthermore, the rate of *C. pneumoniae*-positive DNA in PBMCs decreased after April, especially in September and October, but it increased after November. These data suggest that the prevalence of *C. pneumoniae* DNA in PBMCs has a seasonal variability and are consistent with the previous studies [17, 29, 30]. Further studies are required to clarify whether this seasonal variability is due to a seasonal occurrence of acute *C. pneumoniae* infections in the community and whether this seasonality is a worldwide phenomenon.

**Significance of *C. pneumoniae* DNA in PBMCs.** Although the present study indicates that circulating *C. pneumoniae* DNA is unlikely to be a useful indicator of CAD or ACS, it does not disprove the hypothesis that *C. pneumoniae* may contribute to the development of atherosclerotic diseases. *C. pneumoniae* infection may contribute to the development of atherosclerosis by modulating macrophage-lipoprotein interaction and inducing the expression of various cytokines [32, 33], adhesion molecules [34–38], and chemokines [34–38] in atheroma-associated cells. Furthermore, *C. pneumoniae* infection may produce procoagulants [39, 40] and trigger antibody-mediated endo-

Table 5. Studies describing the seasonal change in the prevalence of Chlamydia pneumoniae DNA in PBMCs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Case group</th>
<th>Case patients</th>
<th>No. of subjects</th>
<th>Prevalence, %</th>
<th>Control group</th>
<th>Control patients</th>
<th>No. of subjects</th>
<th>Prevalence, %</th>
<th>Seasonal variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smieja et al. [17]</td>
<td>2001</td>
<td>Canada</td>
<td>Patients with CAD</td>
<td>187</td>
<td>11.8</td>
<td></td>
<td>Patients with normal angiogram findings</td>
<td>21</td>
<td>9.5</td>
<td>More cases during February to April than during May to October</td>
<td></td>
</tr>
<tr>
<td>Rassu et al. [30]</td>
<td>2001</td>
<td>Italy</td>
<td>Blood donors</td>
<td>169</td>
<td>46.2</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>More cases during February than during October</td>
</tr>
<tr>
<td>Haranaga et al. [31]</td>
<td>2001</td>
<td>United States</td>
<td>Blood donors</td>
<td>237</td>
<td>8.9</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>More cases during August and September than during October and November</td>
</tr>
<tr>
<td>Smieja et al. [29]</td>
<td>2002</td>
<td>Canada</td>
<td>Patients with COPD</td>
<td>100</td>
<td>24.0</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>More cases during winter and spring than during the other seasons</td>
</tr>
<tr>
<td>Present study*</td>
<td></td>
<td>Japan</td>
<td>Patients with CAD</td>
<td>27</td>
<td>59.3b</td>
<td></td>
<td>Patients with normal angiogram findings or no evidence of CAD</td>
<td>32</td>
<td>59.4b</td>
<td>More cases during winter and early spring than during the other seasons</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease.

* The temporal change in the prevalence of *C. pneumoniae* DNA in PBMCs.

b January through March.
theleial cytotoxicity through an immunological cross-reaction [40, 41]. Given the results of our serial PCR tests that circulating \textit{C. pneumoniae} DNA was commonly detected during wintertime and early spring and that the majority (86.4\%) of subjects had $\geq 1$ positive PCR test result, the Japanese population appears to have ample opportunity to acquire vascular \textit{C. pneumoniae} infection that may contribute to the development of atherosclerosis.

\textbf{Limitations of the present study.} The present study has several limitations. First, 38 (43.2\%) of 88 control subjects did not undergo coronary arteriography and may have had significant but asymptomatic coronary artery stenosis. Second, the detection of \textit{C. pneumoniae} DNA by PCR is not definitive proof of the actual presence of \textit{C. pneumoniae} organisms. However, DNA from dead \textit{C. pneumoniae} in tissue specimens is rapidly degraded in vivo [12], and the detection of \textit{C. pneumoniae} in PBMCs by PCR is reproducible and correlates with other detection techniques, such as immunoelectron microscopy and culture [42]. Third, the origin of \textit{C. pneumoniae} DNA in PBMCs remains unclear. Because our subjects had no symptoms of respiratory tract infection within 4 weeks of the obtaining of blood samples, a plausible origin may be systemic dissemination of \textit{C. pneumoniae} after latent acute \textit{C. pneumoniae} infection. Indeed, asymptomatic infections of \textit{C. pneumoniae} might be common [43, 44]. Alternatively, systemic release of \textit{C. pneumoniae} organisms may result from chronic infections in the lung and pharynx [45–47]. Fourth, the prevalence of \textit{C. pneumoniae} DNA in PBMCs in our study was higher than in other reports [16, 17]. Although an outbreak of \textit{C. pneumoniae} infection during the study could have led to the high prevalence of \textit{C. pneumoniae} in PBMCs, there was no evidence of any outbreak in our area during the study. The more likely explanation is that the first samples were obtained in the wintertime and early spring, when seasonal prevalence appears to be at its highest. The difference in the PCR methods used for the detection of \textit{C. pneumoniae} in PBMCs can also be an important cause of the interstudy variability in the prevalence of \textit{C. pneumoniae} DNA in PBMCs. Mahony et al. [28] recently showed that the ompA-nested PCR used in our study was more sensitive for the detection of \textit{C. pneumoniae} DNA in PBMCs and had more reproducible results than did other PCR methods used in previous studies. Our PCR method met the criteria recommended by Dowell et al. [25], and we confirmed the reproducibility and validity of our PCR method. Therefore, we believe that the reliability of our results is high.

\textbf{Conclusions.} The prevalence of \textit{C. pneumoniae} DNA in PBMCs in patients with ACS or stable CAD is comparable to that found in control subjects in the Japanese adult population. The presence of \textit{C. pneumoniae} DNA in PBMCs is unlikely to be an indicator of ACS or CAD. Rather, it appears to be a seasonal phenomenon, with higher a prevalence during wintertime and early spring in Japanese adults.

\textbf{Acknowledgments}

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\textbf{Conflict of interest.} All authors: No conflict.

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