Effects of Interleukin-1 Gene Polymorphisms on the Development of Coronary Artery Disease Associated With Chlamydia Pneumoniae Infection

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OBJECTIVES
This study was done to elucidate the effects of interleukin (IL)-1 gene polymorphisms on coronary artery disease (CAD) associated with Chlamydia pneumoniae (CP) infection.

BACKGROUND
It was suggested that CP was associated with CAD. However, genetic factors involved in CAD associated with CP infection are unknown.

METHODS
We evaluated CP immunoglobulin G (IgG) seropositivity and IL-1β (a C/T transition at −511) and IL-1 receptor antagonist (IL-1Ra) (a variable-number repeat in intron 2) gene polymorphisms in 292 patients undergoing coronary angiography.

RESULTS
Seropositivity for CP was present in 61% of patients with CAD versus 51% without CAD (p = NS). The percentage of patients having IL-1β (−511) C/C genotype and/or IL-1Ra (intron 2) 2- or 3-repeat allele was higher in patients with CAD than without CAD (29% vs. 16%, p < 0.025). To clarify the effects of these CAD-associated variants (IL-1β C/C and/or IL-1Ra 2- or 3-repeat), patients were divided into four groups. A stepwise increase in CAD prevalence was observed depending on CP seropositivity and the variants. Odds ratios (ORs) for CAD were 1.4 in the group with seropositivity alone, 1.7 with the variants alone and 3.8 with seropositivity and the variants. Such variants were associated with CAD in both patients with and without seropositivity. Interestingly, high prevalence of myocardial infarction (MI) was confined to the group with seropositivity and the variants (OR, 2.8). The variants were associated with MI only in patients with CP seropositivity.

CONCLUSIONS
The IL-1 gene polymorphisms were found to play a role in the development of CAD, especially MI, in patients with CP infection. (J Am Coll Cardiol 2001;38:712–7) © 2001 by the American College of Cardiology

Recently, inflammation was suggested to be involved in the pathogenesis of atherosclerosis (1). Infectious agents cause inflammation and may play a role in the development of coronary artery disease (CAD). Chlamydia pneumoniae (CP), one of the common human respiratory pathogens, was frequently reported to be associated with CAD (2–4). However, the potential contribution of CP to CAD is still a matter of debate. Recent prospective studies failed to show any association between CP infection and CAD (5,6). The inflammatory response to CP infection may vary from person to person, and only a small number of individuals develop CAD.

Although CAD is known to have a genetic basis (7–9), genetic factors involved in CAD associated with CP infection are unknown. Interleukin (IL)-1β and IL-1 receptor antagonist (IL-1Ra), which are both cytokines to mediate inflammation, are likely involved in the pathogenesis of CAD (10–12). Recently, a polymorphism of the IL-1β gene (a C/T transition at position −511) and one of the IL-1Ra gene (a variable-number tandem repeat in intron 2) were reported to be associated with CAD (13,14). We hypothesized that these IL-1 gene polymorphisms may have some effects on the development of CAD associated with CP infection.

METHODS
Study subjects. The IL-1β and IL-1Ra gene polymorphisms as well as CP immunoglobulin G (IgG) seropositivity were investigated in 292 consecutive patients who underwent coronary angiography for suspected CAD at National Defense Medical College Hospital from July 1999 to December 2000. Our study was approved by the Ethics Committee of the hospital. After admission, written informed consent was obtained, and blood samples were taken in a fasting state. Coronary angiograms were evaluated blind to the genotype data. Coronary artery disease was defined as at least one coronary artery having >50% luminal diameter stenosis. Myocardial infarction (MI) was confirmed by the documentation of coronary artery stenosis plus either elevations of cardiac enzymes or diagnostic changes on electrocardiograms. Any patients who had coronary artery bypass grafting were excluded, but 32 patients who had previously undergone percutaneous transluminal coronary angioplasty were included.

Serology and genotyping. The cutoff index of serum CP-specific IgG titer was measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (HITAZYME C. pneumoniae, Hitachi Chemical,
Tokyo, Japan). This ELISA used the CP outer membrane complex as a CP-specific antigen. The cutoff index of $\geq 1.10$ was considered seropositive.

We analyzed a polymorphism of the IL-1$\beta$ gene (a C/T base transition at $-511$ in the promoter region) and one of the IL-1Ra gene (variable numbers of an 86-bp tandem repeat in intron 2). Genomic DNA was extracted from blood. Genotyping was performed by polymerase chain reaction (PCR)-base methods as previously reported (15,16). Briefly, the region containing the IL-1$\beta$ polymorphic site was amplified by PCR, and a restriction fragment-length polymorphism analysis was performed after AvaI digestion (15). The region containing the IL-1Ra polymorphic site was also amplified by PCR, and this product was directly analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide (16).

Statistical analysis. Any differences among the groups of patients were evaluated by the unpaired $t$ test and analysis of variance with the Scheffé test for continuous variables and by the chi-square test for categorical variables. Forward stepwise multiple logistic regression analysis was used to elucidate the associations of CP seropositivity and IL-1 gene polymorphisms with CAD. A $p$ value of $<0.05$ was considered to be statistically significant. Results are presented as the mean value $\pm$ SD.

### Table 1. Clinical Characteristics and the Percentages of CP IgG Seropositivity and IL-1 Gene Variants in Patients With and Without CAD

<table>
<thead>
<tr>
<th></th>
<th>CAD (+) ($n = 188$)</th>
<th>CAD (-) ($n = 104$)</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>64 $\pm$ 9</td>
<td>60 $\pm$ 10</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>150 (80%)</td>
<td>58 (56%)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Hypertension (blood pressure $\geq 160/95$ mm Hg or on medication)</td>
<td>92 (49%)</td>
<td>39 (38%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia (TC $&gt;220$ mg/dl or on medication)</td>
<td>91 (48%)</td>
<td>47 (45%)</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>201 $\pm$ 37</td>
<td>204 $\pm$ 38</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>48 $\pm$ 13</td>
<td>57 $\pm$ 16</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>48 (26%)</td>
<td>14 (13%)</td>
<td>$&lt;0.025$</td>
</tr>
<tr>
<td>Smoking</td>
<td>136 (72%)</td>
<td>50 (48%)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>CP IgG titer</td>
<td>1.52 $\pm$ 0.97</td>
<td>1.36 $\pm$ 0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Positive (polymorphism)</td>
<td>115 (61%)</td>
<td>53 (51%)</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1$\beta$ ($-511$) C/C genotype</td>
<td>42 (22%)</td>
<td>14 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1Ra (intron 2) 2- or 3-repeat</td>
<td>14 (7%)</td>
<td>3 (3%)</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1$\beta$ C/C and/or IL-1Ra 2- or 3-repeat</td>
<td>55 (29%)</td>
<td>17 (16%)</td>
<td>$&lt;0.025$</td>
</tr>
</tbody>
</table>

Data are presented as the mean value $\pm$ SD or the number (%) of patients.

CAD = coronary artery disease; CP = Chlamydia pneumoniae; HDL-C = high-density lipoprotein cholesterol; Ig = immunoglobulin; IL = interleukin; IL-1Ra = interleukin-1 receptor antagonist; TC = total cholesterol.

## RESULTS

### Associations of CP seropositivity and IL-1 gene polymorphisms with CAD.

Coronary artery disease was found in 188 patients. Compared with 104 patients without CAD, the 188 patients with CAD were older, predominantly men and had higher rates of diabetes and smoking (Table 1). The prevalence of CP IgG seropositivity was higher in patients with CAD than without CAD (61 vs. 51%), but the difference did not reach statistical significance. As for IL-1 gene polymorphisms, the genotype distributions in patients with and without CAD did not deviate from the Hardy-Weinberg equilibrium. The percentage of patients having IL-1$\beta$ C/C genotype was 22% in patients with CAD versus 13% without CAD. The percentage of patients carrying IL-1Ra 2-repeat or 3-repeat allele was 7% in patients with CAD versus 3% without CAD. The percentage of patients who had the IL-1$\beta$ C/C genotype and/or the IL-1Ra 2- or 3-repeat allele (IL-1 gene variants) was significantly higher in patients with CAD than without CAD (29 vs. 16%, $p < 0.025$). In multivariate analysis, the IL-1 gene variants were found to be associated with CAD, but CP seropositivity was not (Table 2).

### Interaction between CP infection and IL-1 gene polymorphisms.

Of the 292 patients, 168 had CP seropositivity, and 72 had the IL-1$\beta$ C/C genotype and/or IL-1Ra 2- or 3-repeat allele). Between patients with and without CP seropositivity, no significant difference was found in the prevalence of CAD (68 vs. 59%) and MI (35 vs. 28%). To clarify the effects of IL-1 gene variants on CAD associated with CP infection, patients were divided into four groups according to the presence or absence of CP seropositivity and/or IL-1 gene variants. As shown in Figure 1, a stepwise increase in CAD prevalence was seen depending on CP seropositivity and/or IL-1 gene variants. In patients with CP seropositivity, CAD prevalence was higher in those with the variants than in those without the variants (83 vs. 64%, $p < 0.025$).

### Abbreviations and Acronyms

- CAD = coronary artery disease
- CI = confidence interval
- CP = Chlamydia pneumoniae
- ELISA = enzyme-linked immunosorbent assay
- Ig = immunoglobulin
- IL = interleukin
- IL-1Ra = interleukin-1 receptor antagonist
- MI = myocardial infarction
- OR = odds ratio
- PCR = polymerase chain reaction
- TC = total cholesterol
- HDL-C = high-density lipoprotein cholesterol
- IgG = immunoglobulin G
- CP = Chlamydia pneumoniae
0.05), whereas, in patients without seropositivity, the difference did not reach statistical significance between those with and those without such variants (68 vs. 56%). Odds ratios (ORs) for CAD were 1.4 (95% confidence interval [CI], 0.8 to 2.6) in the group with CP seropositivity alone, 2.4 (0.9 to 6.6) in those with the variants alone, and 4.3 (1.5 to 12.4) in those with combined CP seropositivity and variants. In multivariate analysis, the IL-1 gene variants were associated with CAD in both patients with and without CP seropositivity (Table 3).

Of the 188 patients with CAD, 93 had MI. The diagnosis of either acute or old MI was given to 62 or 31 patients, respectively. Although 32 patients with CAD had previously undergone coronary angioplasty, none had MI due to restenosis. Interestingly, the prevalence of MI was characteristically higher in the group with combined CP seropositivity and IL-1 gene variants than in the other three groups (p < 0.05) (Fig. 1), but there was no difference in these three groups. The OR for MI was 2.7 (95% CI, 1.2 to 6.2) in the group with combined seropositivity and variants. In multivariate analysis, the IL-1 gene variants were associated with MI only in patients with CP seropositivity (Table 4).

**DISCUSSION**

We could not find any association of CP seropositivity with CAD, but IL-1 gene variants were associated with CAD. However, a stepwise increase was observed in CAD prevalence depending on positive interaction between CP seropositivity and IL-1 gene variants, and the highest CAD prevalence was seen in patients with combined seropositivity and the variants. These suggest that IL-1 gene polymorphisms appear to influence the susceptibility to the atherogenic effect of CP infection and that patients with such variants are more likely to develop CAD associated with CP infection. Interestingly, the IL-1 gene variants were associated with MI only in patients with CP seropositivity. Thus, IL-1 gene polymorphisms may be modifying the CAD

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**Table 2.** Contributing Factors for Coronary Artery Disease (Multiple Logistic Regression Analysis in 292 Patients)

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>χ²</th>
<th>p Value</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.076</td>
<td>0.02</td>
<td>24.0</td>
<td>&lt;0.001</td>
<td>1.08</td>
<td>(1.05–1.11)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.043</td>
<td>0.01</td>
<td>18.2</td>
<td>&lt;0.001</td>
<td>0.96</td>
<td>(0.94–0.98)</td>
</tr>
<tr>
<td>IL-1 gene variants</td>
<td>0.907</td>
<td>0.35</td>
<td>6.6</td>
<td>&lt;0.005</td>
<td>2.48</td>
<td>(1.24–4.94)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.856</td>
<td>0.35</td>
<td>5.9</td>
<td>&lt;0.025</td>
<td>2.35</td>
<td>(1.18–4.69)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.622</td>
<td>0.32</td>
<td>3.9</td>
<td>&lt;0.05</td>
<td>1.86</td>
<td>(1.00–3.46)</td>
</tr>
</tbody>
</table>

The dependent variable was coronary artery disease. The analysis included age, gender, hyperlipidemia, high density lipoprotein cholesterol (HDL-C), diabetes mellitus, hypertension, smoking, interleukin (IL)-1 gene variants and Chlamydia pneumoniae (CP) immunoglobulin (IgG) seropositivity. *IL-1 gene variants were defined as the IL-1β C/C genotype and/or the interleukin-1 receptor antagonist (IL-1Ra) 2- or 3-repeat allele.

CI = confidence interval; OR = odds ratio; SE = standard error.

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**Figure 1.** Interaction between Chlamydia pneumoniae (CP) seropositivity and interleukin (IL)-1 gene variants in coronary artery disease (CAD) and myocardial infarction (MI). Regarding CAD prevalence, a stepwise increase was seen depending on seropositivity and the variants. In contrast, MI prevalence was characteristically high in the group with combined seropositivity and variants. The IL-1 gene variants were defined as IL-1β C/C genotype and/or IL-1 receptor antagonist 2- or 3-repeat allele.
process associated with CP infection, and patients with such variants are more likely to develop MI in the process of CAD with CP infection.

Infection of CP and CAD. Chlamydia pneumoniae was often reported to be associated with CAD in seroepidemiologic studies (2–4). The association of CP with CAD was strengthened by the detection of the organism within atheroma using direct immunofluorescence and PCR (17,18). A viable organism was also isolated from atheroma (19). Muhlestein et al. (20) showed that CP infection accelerates atherosclerosis in a rabbit model. However, the potential contribution of CP to CAD remains controversial. Two prospective studies (5,6) and one recent case-control study (21) failed to show any association between CP infection and CAD. We also found no association between CP seropositivity and CAD. Individuals may vary in their inflammatory response to CP infection, and only certain individuals may be susceptible to the atherogenic effects of CP infection, thus leading to the development of CAD.

The IL-1 gene polymorphisms. Recently, Lacoviello et al. (13) reported IL-1β C/C genotype at −511 to be associated with MI. Nemetz et al. (22) reported that, in ulcerative colitis, the IL-1β C/C genotype was associated with severe bleeding and surgery and suggested that this polymorphism plays a role in determining the course and severity of the disease. However, the functional effect of this polymorphism remains unclear. Santtila et al. (23) found no significant difference in IL-1β production in vitro between alleles.

Francis et al. (14) reported that IL-1Ra 2-repeat allele in intron 2 was associated with CAD, especially single-vessel disease. They suggested that disease-modifying gene polymorphisms may determine the pattern of CAD. This polymorphism has five alleles, from 2 to 6 repeats, and the 4-repeat allele is most common. The 2-repeat allele was suggested to be sufficient to affect disease such as ulcerative colitis, alopecia and diabetic nephropathy (26–28). Moreover, the presence of one copy of the 2-repeat allele was suggested to be sufficient to affect these diseases (27).

In our study, the IL-1β and IL-1Ra gene polymorphisms were not independent factors for CAD. However, the percentage of patients having IL-1β C/C genotype and/or IL-1Ra 2- or 3-repeat allele was higher in CAD, and these variants were associated with CAD. Moreover, these variants were associated with MI in patients with CP seropositivity, suggesting that such IL-1 gene polymorphisms modify the process of CAD associated with CP infection, thereby leading to the development of MI.

### Table 3. Contributing Factors for Coronary Artery Disease (Multiple Logistic Regression Analysis in 168 Patients With CP Seropositivity and 124 Without CP Seropositivity)

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>χ²</th>
<th>p Value</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP seropositivity (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.044</td>
<td>0.01</td>
<td>9.9</td>
<td>&lt;0.005</td>
<td>0.96</td>
<td>(0.93–0.98)</td>
</tr>
<tr>
<td>Age</td>
<td>0.049</td>
<td>0.02</td>
<td>6.2</td>
<td>&lt;0.025</td>
<td>1.05</td>
<td>(1.01–1.09)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.950</td>
<td>0.43</td>
<td>4.9</td>
<td>&lt;0.05</td>
<td>2.59</td>
<td>(1.11–6.02)</td>
</tr>
<tr>
<td>IL-1 gene variants*</td>
<td>1.038</td>
<td>0.49</td>
<td>4.4</td>
<td>&lt;0.05</td>
<td>2.82</td>
<td>(1.07–7.42)</td>
</tr>
<tr>
<td>CP seropositivity (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.114</td>
<td>0.03</td>
<td>17.7</td>
<td>&lt;0.001</td>
<td>1.12</td>
<td>(1.06–1.18)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.948</td>
<td>0.50</td>
<td>15.1</td>
<td>&lt;0.001</td>
<td>7.01</td>
<td>(2.62–18.8)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.046</td>
<td>0.02</td>
<td>8.0</td>
<td>&lt;0.005</td>
<td>0.96</td>
<td>(0.93–0.99)</td>
</tr>
<tr>
<td>IL-1 gene variants*</td>
<td>1.075</td>
<td>0.55</td>
<td>3.8</td>
<td>&lt;0.05</td>
<td>2.93</td>
<td>(1.00–8.54)</td>
</tr>
</tbody>
</table>

The dependent variable was coronary artery disease. The analysis included age, gender, hyperlipidemia, high density lipoprotein cholesterol (HDL-C), diabetes mellitus, hypertension, smoking, and interleukin (IL)-1 gene variants. *IL-1 gene variants were defined as the IL-1β C/C genotype and/or the interleukin-1 receptor antagonist (IL-1Ra) 2- or 3-repeat allele.

### Table 4. Contributing Factors for Myocardial Infarction (Multiple Logistic Regression Analysis in 168 Patients With CP Seropositivity and 124 Without CP Seropositivity)

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>χ²</th>
<th>p Value</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP seropositivity (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1 gene variants*</td>
<td>1.028</td>
<td>0.38</td>
<td>7.5</td>
<td>&lt;0.025</td>
<td>2.80</td>
<td>(1.34–5.84)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.031</td>
<td>0.01</td>
<td>5.1</td>
<td>&lt;0.025</td>
<td>0.97</td>
<td>(0.94–1.00)</td>
</tr>
<tr>
<td>CP seropositivity (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>−0.075</td>
<td>0.02</td>
<td>13.6</td>
<td>&lt;0.001</td>
<td>0.93</td>
<td>(0.90–0.95)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.993</td>
<td>0.49</td>
<td>4.1</td>
<td>&lt;0.05</td>
<td>2.70</td>
<td>(1.03–7.09)</td>
</tr>
</tbody>
</table>

The dependent variable was myocardial infarction. The analysis included age, gender, hyperlipidemia, high density lipoprotein cholesterol (HDL-C), diabetes mellitus, hypertension, smoking, and interleukin (IL)-1 gene variants. *IL-1 gene variants were defined as the IL-1β C/C genotype and/or the interleukin-1 receptor antagonist (IL-1Ra) 2- or 3-repeat allele.

CI = confidence interval; CP = Chlamydia pneumoniae; OR = odds ratio; SE = standard error.
Interaction between CP infection and IL-1 gene polymorphisms. Genetic factors involved in CAD associated with CP infection are unknown. Only one study (29) showed HLA-DR-13a or -17 genotype in combination with high lipoprotein (a) levels and CP seropositivity to be more frequent in CAD. However, the difference compared with controls was only significant in men with a lipoprotein (a) level of ≥120 mg/dl. We showed the presence of a stepwise increase in CAD prevalence depending on the positive interaction between CP seropositivity and IL-1 gene variants, whereas, in addition, the highest CAD prevalence occurred in patients with combined seropositivity and variants. Our results suggest that the joint effect of CP seropositivity and IL-1 gene variants seems to be additive and that IL-1 gene polymorphisms influence susceptibility to the atherogenic effects of CP infection. These findings may provide a potential explanation for conflicting evidence regarding the role of CP infection in CAD, because genetically determined susceptibility to its atherogenic effect may be an important factor in determining whether or not it contributes to CAD.

We also showed that IL-1 gene variants were associated with MI only in patients with CP seropositivity. Although patients without CP seropositivity may have some other infections, this modifying effect on the CAD process was confined to patients with CP infection. Thus, IL-1 gene polymorphisms may characteristically modify the process of CAD associated with CP infection, leading to the development of MI.

Study limitations. First, our study is cross-sectional; such a study cannot establish causality. It shows some association and is hypothesis-generating. Second, we did not have healthy controls. We studied consecutive patients undergoing angiography, who were divided into two groups with and without CAD. Some patients without CAD had mild, but not significant, stenosis. These may have caused some selection bias and may have confounded the results. Third, our study was in a Japanese population. The frequencies of IL-1β C allele and IL-1Ra 2-repeat allele in healthy Japanese were reported to be 0.54 and 0.03, respectively (30), which were lower than those in healthy Caucasians (0.62 to 0.65 and 0.24) (22,26). Our results may not be applicable to other ethnic populations. However, the percentage of individuals susceptible to CAD associated with CP infection may be higher in Caucasians than in Japanese.

Conclusions. The IL-1 gene polymorphisms were found to play a role in the development of CAD, especially MI, in patients with CP infection. The joint effect of CP seropositivity and IL-1 gene variants on the development of CAD seems to be additive, but the modifying effect of these variants on CAD process may be limited only to patients with CP seropositivity, leading to the development of MI.

REFERENCES


