Functional Polymorphisms of the Cyclooxygenase-2 Gene and Risk for Chronic Obstructive Pulmonary Disease in Japanese Population

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation. Cyclooxygenase-2 (COX-2) plays a very important role in the progress of inflammation. Several potentially functional polymorphisms of the COX-2 gene were identified and proposed to be associated with COPD susceptibility. In this study, we aimed to investigate a possible association between COX-2 (-765G>C; -1195G>A) polymorphisms and the risk of COPD in the Japanese population. COX-2 gene polymorphisms were identified by polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) in 103 Japanese patients with COPD and 129 healthy controls. The distributions of genotypes and alleles were compared by using the χ² test. The odds ratios (OR) and 95% confidence intervals (95%CI) were calculated by a logistic regression analysis. The frequencies of the GG, GC, CC genotypes of COX-2-765G>C and GG, GA, AA genotypes of COX-2-1195G>A in the COPD group were 94.2%, 5.8%, 0.0% and 40.8%, 44.6%, respectively. These frequencies in control group were 89.1%, 10.9%, 0.0% and 23.3%, 44.9%, 31.8%, respectively. The COX-2-1195AA was more prevalent in the COPD group (44.6%) than the control group (31.8%). The distribution of the -765C was higher in the control group (5.5%) in comparison to the COPD group (2.9%). In conclusion, the COX-2-1195AA genotype was associated with an increased risk for COPD in the Japanese population.

Key words: Japanese, COX-2, polymorphisms, risk of COPD

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide [1]. The World Health Organization estimates that more than 3 million people died of COPD in 2005, which corresponds to 5% of all deaths globally [2]. COPD is an inflammatory disease characterized by the progressive deterioration of pulmonary function and increasing airway obstruction. Accumulating studies show several genetic risk factors are associated with susceptibility to COPD, such as α1-antitrypsin, α1-antichymotrypsin, cystic fibrosis transmembrane regulator, vitamin D-binding protein, α2-macroglobulin, cytochrome P4501A1, blood group antigens, human leukocyte antigen, immunoglobulin deficiency, and haptoglobin [3].

Cyclooxygenase (COX) is a key enzyme in the conversion of arachidonic acid into prostaglandins. COX-2 is an induced isofrom which is characteristic of cells involved in inflammatory processes [4]. The over-expression of COX-2 is linked to enhancement of cellular proliferation, promotion of angiogenesis, inhibition of apoptosis, stimulation of invasion, and suppression of immune responses [5, 6]. Several common single nucleotide polymorphisms (SNPs) of the COX-2 gene are reportedly
functional and associated with human tumors of various organs [7-13]. On the other hand, a few studies have so far suggested that COX-2 plays a biologically important role in the inflammation of respiratory disease [14-16].

The purpose of this study was to evaluate whether the COX-2 -765G>C or -1195G>A polymorphisms are associated with susceptibility to COPD.

MATERIALS AND METHODS

Patients and Samples

One hundred and three patients with COPD and 129 healthy controls took part in this study. Patients were recruited from the Department of Internal Medicine, Division of Clinical Oncology & Respiratory Medicine, Shimane University Hospital (Shimane, Japan). COPD was diagnosed based on clinical history, physical examination, and pulmonary functional tests, according to the GOLD criteria [1]. The patients had an FEV1/FVC ratio of <70% and FEV1 of <80% of predicted values. The control subjects were sex- and age-matched, and visited the same hospitals for a routine health checkup. Control subjects had normal pulmonary function tests (FEV1/FVC > 70% and FEV1 > 80% of predicted values) with no history of airway disease or abnormal findings on chest radiography.

All subjects gave written informed consent for participation in the study. The study protocol was approved by local ethics committee.

DNA preparation and genotype determination

Peripheral blood samples were collected from each study subject into EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood samples using a DNA extractor WB-Rapid kit (Wako, Osaka, Japan). PCR-RFLP was used for the analysis of COX-2 polymorphisms. The PCR was carried out in a reaction mixture of 50 uL containing 2.5 U Taq and 1 uL template DNA with a concentration of approximately 50-150 ng/ml. The COX-2 -765G>C polymorphism was determined using primers 5'-ATT CTG GCC ATC GCC GCT TC-3' (forward) and 5'-GCC CTT CAT AGG AGA TAC TGG-3' (reverse). The PCR cycling conditions for the -765G>C and -1195G>A polymorphisms analyses were: 5 min at 96°C followed by 32 cycles of 20 sec at 96°C, 30 sec at 52°C, and 30 sec at 72°C, with a final extension of 72°C for 6 min. The 157 bp PCR product of -765G>C was digested using Bsh1236I into two fragments of 134 bp and 23 bp for the 765G allele. The 273 bp product of the -1195G>A PCR product was digested with PvuII into 220 bp and 53 bp fragments for the -1195G allele. The digested products were separated by electrophoresis in 2% agarose gels stained with ethidium bromide under UV light (Figs 1 and 2). The PCR products were sequenced directly at random to rule out the possibility of PCR errors.

Fig. 1. Patterns of PCR-RFLP for COX-2-765G>C. M: 100 bp DNA ladder; Line 1, 2, 3, 5: Homozygous -765GG genotype; Line 4: Heterozygous -765GC genotype; Line 6: Positive control of homozygous -765CC genotype; Line 7: Positive control of homozygous -765GG genotype. The PCR product was digested with Bsh1236I, which cleaved wild genotype G into 134 bp and 23 bp fragments, variant allele C remained an uncleaved 157 bp fragment.

Fig. 2. Patterns of PCR-RFLP for COX-2-1195G>A. M: 100 bp DNA ladder; Line 1, 2, 3: Homozygous -1195GG genotype; Line 4, 5, 6, 7: Homozygous -1195AA genotype; Line 8, 9, 10, 11: Heterozygous -1195GA genotype. The PCR product was digested with PvuII, which cleaved wild genotype G into 220 bp and 53 bp fragments, variant allele A remained an uncleaved 273 bp fragment.
**Statistical analysis**

The Statistical Package for Social Sciences software package (Version 17.0) was used for all statistical analyses. Data are expressed as the mean ± standard deviation (SD) or numbers (%) of subjects. The allele and genotype frequencies of the patients and controls were compared with the χ² test. The analysis of association between COX-2 polymorphisms and COPD was based on the 95% CI for the disease odds ratio (OR) calculated. Logistic regression was used to assess the association between the genotype and the risk of COPD. The Hardy-Weinberg equilibrium was assessed for the distributions of genotype in each group. *P* <0.05 was considered to be statistically significant.

**RESULTS**

**Baseline clinical characteristics of study subjects**

Table 1 summarizes the demographic characteristics and relevant clinical parameters of all study subjects. The age and gender distributions were similar between COPD and control groups. Non-smokers were more in controls than COPD group. As expected, the FEV1, % pred and FEV1/FVC, % in the controls were higher than those in the COPD group.

<table>
<thead>
<tr>
<th></th>
<th>COPD (n=103)</th>
<th>Controls (n=129)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.5 ± 6.2</td>
<td>58.4 ± 9.1</td>
<td>0.420</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>100 (97.1)</td>
<td>125 (92.4)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3 (2.9)</td>
<td>4 (7.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2 (1.9)</td>
<td>33 (25.6)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>101 (98.1)</td>
<td>96 (74.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1, %pred</td>
<td>51.4 ± 0.9</td>
<td>99.0 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>48.8 ± 1.1</td>
<td>85.4 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 1. Baseline clinical characteristics of study subjects.

Values represent the number (%) of subjects or the mean ± SD. FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.

**Analysis of COX-2-765G>C and -1195G>A genotypes**

Table 2 summarizes the SNP distributions and allele frequencies. All genotypic distributions accorded with the Hardy-Weinberg equilibrium (*P* >0.05).

The frequencies of the COX-2-765GG, GC, and CC genotypes among the COPD group were 94.2%, 5.8%, and 0.0%, respectively, in comparison to 89.1%, 10.9%, and 0.0%, respectively, in the controls. The -765C allele was quite scarce, with no CC homozygote in either COPD or control subjects. The GC genotype was lower in COPD than control subjects (5.8% versus 10.9%), but the decreased risk was not significant (adjusted OR=0.509, 95%CI 0.181-1.376, *P* = 0.223).

The frequencies of the COX-2-1195GG, GA, and AA genotypes in COPD were 14.6%, 40.8%, and 44.6%, respectively, in comparison to 23.3%, 44.9%, and 31.8%, respectively, in the controls. The COX-2-1195G>A polymorphism was more general, with similar distributions of -1195GG and GA genotypes between the two groups, but a higher frequency of the -1195AA homozygote in COPD subjects than controls (44.6% versus 31.8%). The individuals who had the -1195AA genotype had an increased risk for COPD (adjusted OR=2.246, 95%CI 1.064-4.759, *P* =0.041).
The present study is the first one to assess the role of the COX-2 gene polymorphisms in COPD in the Japanese population. There was no significant difference in the genotype distribution and the allele frequency of COX-2-765G>C among the COPD and control groups. The -765GG genotype was more prevalent in the Japanese population, the frequency of -765GG genotype was 94.2% in COPD patients and 89.1% in controls. No -765CC homozygosity was found in both groups. The distribution of the -765C allele was more in controls than COPD subjects (5.5% versus 2.9%), but the increase was not statistically significant (adjusted OR=0.478, 95%CI 0.126-1.988, P=0.445). The reported frequency of -765C allele seems to vary among different ethnic populations, it is 21%-32% in Caucasian and African Americans [17], 14%-28% in Europeans [18-21], 16% in healthy Hindus [22], 5% in Singaporeans [9], 2.3% in Japanese [23]. In our study, the frequency of C allele was greater than the reported in Japanese, as described above, the discrepancy could be explained by diverse geographical area. Indeed, the complex mechanisms of genovariation are unclear, and the effects of external factors could not be excluded. There are many studies about the effect of COX-2-765CC genotype or -765C allele, but the conclusions are conflicting. For instance, individuals carrying the -765C allele have lower risks of myocardial infarction and ischemic stroke in Italy [17], and have a decreased risk of Alzheimer’s disease in the USA [24], while another study showed the -765CC genotype increases the risk of asthma and the PGE2 production is 10 times higher than the GG group [20]. These outcomes indicate that the COX-2-765G>C polymorphism may have a significantly different modulating effects on various disease phenotypes in different races and regions.

<table>
<thead>
<tr>
<th>SNP</th>
<th>COPD (n=103)</th>
<th>control (n=129)</th>
<th>P value</th>
<th>OR (95%CI)</th>
<th>OR* (95%CI)</th>
<th>P value</th>
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<tr>
<td>765G&gt;C</td>
<td></td>
<td></td>
<td></td>
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<td>Reference</td>
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<tr>
<td>GG</td>
<td>97 (94.2)</td>
<td>115 (89.1)</td>
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<tr>
<td>GC</td>
<td>6 (5.8)</td>
<td>14 (10.9)</td>
<td>0.240</td>
<td>0.508 (0.188-1.373)</td>
<td>0.509 (0.181-1.376)</td>
<td>0.223</td>
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<tr>
<td>CC</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>Allele</td>
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<td></td>
<td></td>
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<tr>
<td>G</td>
<td>97.1%</td>
<td>94.5%</td>
<td>Reference</td>
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<tr>
<td>C</td>
<td>2.9%</td>
<td>5.5%</td>
<td>0.498</td>
<td>0.490 (0.119-2.015)</td>
<td>0.478 (0.126-1.988)</td>
<td>0.445</td>
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<td>HWE (x2/P)</td>
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<td>1195G&gt;A</td>
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<td>GG</td>
<td>15(14.6)</td>
<td>30(23.3)</td>
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<tr>
<td>GA</td>
<td>42 (40.8)</td>
<td>58(44.9)</td>
<td>0.362</td>
<td>1.448 (0.694-3.024)</td>
<td>1.441 (0.690-3.029)</td>
<td>0.357</td>
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<tr>
<td>AA</td>
<td>46 (44.6)</td>
<td>41 (31.8)</td>
<td>0.043</td>
<td>2.244 (1.061-4.747)</td>
<td>2.246 (1.064-4.759)</td>
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</tr>
<tr>
<td>G</td>
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<td>45.8%</td>
<td>Reference</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>65.0%</td>
<td>54.2%</td>
<td>0.150</td>
<td>1.582 (0.896-2.794)</td>
<td>1.577 (0.885-2.808)</td>
<td>0.147</td>
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<td>HWE (x2/P)</td>
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</table>

HWE, Hardy-Weinberg equilibrium test; OR, odds ratio; 95%CI, 95% confidence interval; *Adjusted for age, gender and smoking history.
NS, not significant.

**DISCUSSION**

The present study is the first one to assess the role of the COX-2 gene polymorphisms in COPD in the Japanese population. There was no significant difference in the genotype distribution and the allele frequency of COX-2-765G>C among the COPD and control groups. The -765GG genotype was more prevalent in the Japanese population, the frequency of -765GG genotype was 94.2% in COPD patients and 89.1% in controls. No -765CC homozygosity was found in both groups. The distribution of the -765C allele was more in controls than COPD subjects (5.5% versus 2.9%), but the increase was not statistically significant (adjusted OR=0.478, 95%CI 0.126-1.988, P=0.445). The reported frequency of -765C allele seems to vary among different ethnic populations, it is 21%-32% in Caucasian and African Americans [17], 14%-28% in Europeans [18-21], 16% in healthy Hindus [22], 5% in Singaporeans [9], 2.3% in Japanese [23]. In our study, the frequency of C allele was greater than the reported in Japanese, as described above, the discrepancy could be explained by diverse geographical area. Indeed, the complex mechanisms of genovariation are unclear, and the effects of external factors could not be excluded. There are many studies about the effect of COX-2-765CC genotype or -765C allele, but the conclusions are conflicting. For instance, individuals carrying the -765C allele have lower risks of myocardial infarction and ischemic stroke in Italy [17], and have a decreased risk of Alzheimer’s disease in the USA [24], while another study showed the -765CC genotype increases the risk of asthma and the PGE2 production is 10 times higher than the GG group [20]. These outcomes indicate that the COX-2-765G>C polymorphism may have a significantly different modulating effects on various disease phenotypes in different races and regions.
The current study was also the first that addressed the correlation between COPD and COX-2-1195G>A in the Japanese population. Individuals carrying -1195AA genotype had an increased risk for COPD. Polymorphism in COX-2-1195G>A are fairly common in humans. The distributions of -1195GG, GA and allele G, A were similar in the COPD and control groups, but the -1195AA genotype was higher in COPD subjects than controls (44.6% versus 31.8%), suggesting that the -1195AA was a risk genotype for COPD (P=0.041, adjusted OR=2.246, 95%CI 1.064-4.759). A possible mechanism for this effect is that the -1195A allele creates a c-Myb binding site to upregulate the gene expression, thus the -1195A allele is a risk factor by increasing the promoter activity; this possibility is consistent with other studies [8, 10, 25-27].

In conclusion, the current findings suggested that COPD was not associated with the COX-2-765G>C polymorphism, but not the COX-2-1195G>A polymorphism, the -1195AA genotype was a risk factor for COPD. Further studies with a larger sample size are necessary to clarify the role of COX-2 in COPD among different geographic areas and races.

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