Genetic polymorphism of the angiotensin-converting enzyme (ACE) in asthmatic patients


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Angiotensin-converting enzyme (ACE) inactivates bradykinin, substance P and neuropeptide A, which are believed to play important roles in the pathogenesis of asthma, especially in neurogenic inflammation. It has recently been shown that an insertion (I)/deletion (D) polymorphism in the ACE gene accounts for variation in serum ACE levels. There are thus three genotypes (insertion homozygote, II; deletion homozygote, DD; heterozygotes, DI). The serum ACE level with the DD type is reported to be about double that of the II type and intermediate in the DI case. In the present study, we examined whether asthma is linked with this ACE gene polymorphism.

Seventy-one patients with asthma (27 males and 44 females) and 142 sex- and age-matched healthy controls were determined for their genotype by the polymerase chain reaction (PCR) method.

Twenty-five asthmatics demonstrated the II type (35.2%), 37 the DI type (52.1%), and nine the DD type (12.7%). There were no significant differences in the distributions of genotypes and serum ACE levels between healthy controls and patients. No significant differences were evident in serum IgE levels, age at onset, proportion of atopic type patients and severity of asthma among the three genotypes.

We did not find any association between asthma and the ACE gene polymorphism in this study.

Introduction

The angiotensin-converting enzyme (ACE) (kininase II, peptidyl dipeptidase A; EC 3.4.15.1) inactivates bradykinin and tachykinins such as substance P (SP) and neuropeptide A (NKA). These are considered to play important roles in the pathogenesis of asthma, especially in neurogenic inflammation. Bradykinin causes bronchoconstriction, enhances vascular permeability, leads to mucosal oedema, and increases the production of mucus by direct effects or stimulating release of neuropeptides, such as tachykinins from C-fibres (1-5). Patients with asthma are reported to show bronchial hyperreactivity to bradykinin, relative to healthy subjects (6). Tachykinins are inducers of airway smooth muscle constriction, bronchial oedema, extravasation of plasma, and mucus hypersecretion, acting as important mediators of neurogenic inflammation (1-4,7).

A number of investigators have reported that ACE inhibitors increase the bronchial responsiveness of patients with asthma (8,9). Furthermore, it is well known that they often cause non-productive coughing as a side-effect, possibly due to bradykinin influence on the airways. Thus changes in the enzyme may play a role in asthma pathogenesis.

It has recently been shown that the ACE gene contains a polymorphism based on the presence [insertion (I)] or absence [deletion (D)] of a nonsense DNA fragment (10). The polymorphism is located in intron 16, so the ACE itself does not differ due to the genotypic variation, but the polymorphism accounts for 47% of the total phenotypic variance in serum ACE levels. There are three genotypes: deletion homozygotes, DD; insertion homozygotes, II; and heterozygotes, DI. The serum ACE level with the DD type is reported to be about double that of the II type and that of the DI type is intermediate. The DD genotype has been reported to be a genetic risk factor for myocardial infarction, left ventricular hypertrophy (11,12), but the effects of ACE gene polymorphism in terms of susceptibility to development of asthma remain unclear (13). We therefore examined genes of asthmatic patients and controls to determine whether any association may exist.
Table 1. Subjects

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Sex (F/M)</th>
<th>Mean age (year ± SEM)</th>
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</thead>
<tbody>
<tr>
<td>Asthmatic patients</td>
<td>71</td>
<td>44/27</td>
<td>52.0 ± 2.0</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>142</td>
<td>82/54</td>
<td>51.9 ± 1.2</td>
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</table>

Materials and Methods

SUBJECTS

Seventy-one asthmatic patients living in central Japan were examined. All satisfied the American Thoracic Society criteria for asthma (14). We selected those subjects who had no cardiomegaly on chest radiography, normal findings on ECG, normal findings on UCG performed by cardiac specialists, FEV₁ > 70% on spirogram, and normal findings on chest computed tomography (CT) evaluated by a radiologist, to exclude cardiogenic disease and pulmonary emphysema. Twenty-seven of the cases were males and 44 were females. They had a mean age of 52.0 ± 2.0 years (mean ± SEM). Forty-nine of the 71 patients had atopic, and 22 non-atopic disease. According to the severity classification of the International Consensus Report on diagnosis and treatment of asthma, 25 cases were mild; 37 were moderate and nine were severe (15).

As controls, 142 sex- and age-matched, unrelated healthy subjects from the same area of Japan were selected. They comprised 54 males and 88 females with a mean age of 51.9 ± 1.2 years. They did not have any past histories of pulmonary disease and atopic disease or any abnormalities on physical examination, chest radiography, spirometry, blood pressure testing, ECG, urinalysis and routine laboratory blood testing. None was receiving medication at the time of evaluation. Informed consent was obtained from all the patients and healthy controls (Table 1). The percentages of current and past cigarette smokers in asthmatic patients were 14.8% (10/71) and 11.3% (8/71), and in healthy subjects were 21.8% (31/142) and 9.9% (14/142), respectively.

DETERMINATION OF ACE GENOTYPES

The D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene and size fractionation and visualization by electrophoresis. DNA was extracted from peripheral leukocytes with standard techniques. PCR was performed with 20 pmol of each primer: sense oligo 5'-CTGGAGACCACTCCCATTTACTC-3' and anti-sense oligo: 5'-GATGGCCATCACATTGTCAGAT-3'. The DNA was amplified for 30 cycles with denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min. PCR products were electrophoresed in 2% agarose gels with 300 ng ethidium bromide per mm. The amplification products of the D and I alleles were identified by 300-nm UV trans-illumination as distinct bands (D allele: 191 bp; I allele: 478 bp) [Fig. 1(a)].

As the D allele in heterozygous samples is preferentially amplified, each sample found to have the DD genotype was...
Table 2. Results of genotyping

<table>
<thead>
<tr>
<th></th>
<th>II (%)</th>
<th>DI (%)</th>
<th>DD (%)</th>
<th>I allele/D allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>n=142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthmatic patients</td>
<td>n=71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>57 (40.1)</td>
<td>69 (48.6)</td>
<td>16 (11.3)</td>
<td>0-644/0-356</td>
</tr>
<tr>
<td></td>
<td>25 (35.2)</td>
<td>37 (52.1)</td>
<td>9 (12.7)</td>
<td>0-613/0-387</td>
</tr>
</tbody>
</table>

No significant differences in the genotype distribution ($\chi^2=0.497$, d.f.=$2$, $P=0.780$) or the allele frequencies ($\chi^2=0.410$, d.f.=$1$, $P=0.522$) were observed between healthy controls and asthmatic patients.

subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence (sense 5a, 5'TGGGACACAGGCGCCGACTAC'; antisense 5c, 5'TCGCCAGCCCTCCCATGCCCCATAA'), with identical PCR conditions except for an annealing temperature of 67°C. The reaction yields a 335-bp amplicon only in the presence of an I allele, and no product in samples homozygous for DD (18,19). This procedure correctly identified about 2% of samples (3/152) with the DI genotype that were misclassified as DD with the insertion-spanning primer (Fig. 1(b)). The PCR results were scored by two independent investigators, blind as to whether the sample was from an asthmatic patient or a control.

SERUM IGE AND ACE LEVEL MEASUREMENT
Serum ACE levels were measured by a colorimetric method (colorimetric assay kit, Fujizoki Assay, Tokyo, Japan) using p-hydroxyhippuryl-L-histidyl-L-leucine as the substrate (20). Serum IgE levels were measured by the radioimmunosorbent test (RIST).

STATISTICAL ANALYSIS
Values are expressed as means ± SEM. The allele ratios and genotype distributions of asthmatic patients and healthy controls, and variation in clinical manifestation of the disease among the three genotypes were analysed with the chi-squared test. Analysis of serum ACE and IgE levels was performed using the Mann-Whitney U test for comparison of the two groups, and the Kruskal-Wallis test for the three groups (21). A P-value <0.05 was considered significant.

Results
GENOTYPE DISTRIBUTION
Of the 142 healthy controls, 57 had the II genotype, 69 the DI type and 16 the DD type. The I allele/D allele (I/D) ratio was 0.644±0.356. Of the 71 patients with asthma, 25 were type II, 37 were DI and nine were DD. The I/D ratio was 0.613±0.387. The observed genotype distributions were in agreement with the Hardy-Weinberg proportion. No significant differences in genotype distribution ($\chi^2=0.497$, d.f.=$2$, $P=0.780$) or the allele frequencies ($\chi^2=0.410$, d.f.=$1$, $P=0.522$) were observed between healthy subjects and asthmatic patients (Table 2).

CLINICAL MANIFESTATION AND ACE GENE POLYMORPHISM
No significant differences were found among the three genotypes regarding age at onset of asthma ($P=0.957$), for the proportion of patients with atopic and non-atopic type disease ($\chi^2=0.164$, d.f.=$2$, $P=0.921$) or for the severity of asthma ($\chi^2=5.70$, d.f.=$4$, $P=0.223$) (Table 3).

SERUM IGE AND ACE LEVELS
In healthy controls, the average serum ACE levels of II, DI and DD individuals were 11.3±0.5, 13.7±0.5 and 17.1±0.8 IU 1−1, respectively. For 70 of the 71 asthmatic patients, serum ACE levels were measured, the average values for the II, DI and DD types being 11.5±1.0, 13.5±0.6 and 14.5±1.0 (IU 1−1), respectively. Significant differences among the three genotypes were found for both asthmatic patients ($P<0.05$) and normal controls ($P<0.0001$). However, the serum ACE levels of asthmatic patients did not significantly differ from those of healthy controls in each genotype (Table 4). There were no significant differences in the serum IgE levels among the three genotypes in asthmatic patients ($P=0.907$).

Discussion
ACE is a widely distributed zinc-metalloproteinase, occurring, e.g., as a membrane-bound ectoenzyme on the surface of vascular endothelial cells and renal epithelial cells, and as a circulating enzyme in plasma. The ACE gene spans 21 k, is located on the 17th chromosome q23, and consists of 26 exons and 25 introns. The polymorphism exists in intron 16. The length of the insertion is 287 bp, and it is a repetition of a meaningless Alu family configuration (22,23). It has not been resolved why the polymorphism influences the serum ACE level but some authors have suggested that the insertion/deletion may be in linkage disequilibrium with regulatory elements of the ACE gene, or that the insertion itself might modify the splicing process of the ACE precursor mRNA by interfering with the lariat formation step (10,24).
Table 3. Clinical manifestation of asthma and genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>II (%)</th>
<th>DI (%)</th>
<th>DD (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=25</td>
<td>n=36</td>
<td>n=9</td>
<td>n=71</td>
</tr>
<tr>
<td>Age at onset of asthma (years)</td>
<td>37.8 ± 4.2</td>
<td>37.6 ± 3.8</td>
<td>39.9 ± 6.9</td>
<td>38.0 ± 2.6</td>
</tr>
<tr>
<td>Atopic</td>
<td>18 (72.0%)</td>
<td>25 (67.6%)</td>
<td>6 (66.7%)</td>
<td>49</td>
</tr>
<tr>
<td>Non-atopic</td>
<td>7 (28.0%)</td>
<td>12 (32.4%)</td>
<td>3 (33.4%)</td>
<td>22</td>
</tr>
<tr>
<td>Mild</td>
<td>12 (48.0%)</td>
<td>10 (27.0%)</td>
<td>1 (11.1%)</td>
<td>23</td>
</tr>
<tr>
<td>Moderate</td>
<td>11 (44.0%)</td>
<td>21 (56.8%)</td>
<td>7 (77.8%)</td>
<td>39</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (8.0%)</td>
<td>6 (16.2%)</td>
<td>1 (11.1%)</td>
<td>9</td>
</tr>
</tbody>
</table>

All 71 asthmatic patients were evaluated for clinical manifestation. No significant difference in age at onset (P=0.957), frequencies of atopic type (χ²=0.164, d.f.=2, P=0.921) and severity of asthma (χ²=5.70, d.f.=4, P=0.223) were noted among the three genotypes. The severity was determined by the classification of the International Consensus Report on diagnosis and treatment of asthma.

Table 4. Serum ACE and IgE levels

<table>
<thead>
<tr>
<th>Serum ACE level (IU l⁻¹)</th>
<th>Serum IgE level (IU ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>Asthmatic patients</td>
</tr>
<tr>
<td>II n=57</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>DI n=69</td>
<td>13.7 ± 0.5</td>
</tr>
<tr>
<td>DD n=16</td>
<td>17.1 ± 0.8</td>
</tr>
<tr>
<td>Total n=142</td>
<td>13.1 ± 0.4</td>
</tr>
<tr>
<td>P-value</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

The serum ACE level was measured for 70 of 71 asthmatic patients and for all 142 healthy controls. Significant differences were found among the three genotypes both for asthmatic patients and healthy controls, but not between these two groups. There were no significant differences in serum IgE level among the three genotypes.

Bradykinin and tachykinins are important mediators for neurogenic inflammation. Bradykinin is inactivated by ACE (kinase II) and kinase I (carboxypeptidase N), and tachykinins are mainly degraded by ACE and neutral endopeptidase (NEP, EC 3.4.24.11, enkephalinase) (1-5). Therefore, alteration in ACE may play a role in asthma. Non-productive coughing caused by ACE inhibitors is reported to be more frequent with the II genotype than in the other types (25). In addition, Morice et al. showed an increase in the sensitivity of the cough reflex to stimulation with distilled water in II genotype individuals (26). We therefore hypothesized that low ACE activity would predispose to development of asthma, and the II type may be a genetic risk factor for the disease due to the lower ability of inactivating bradykinin and tachykinins. In this study, however, we did not find any significant associations between the ACE gene polymorphism and asthma. Moreover, the ACE genotype distribution of asthmatic patients did not significantly differ from that of 207 sarcoidosis patients in our previous study (χ²=1.248, d.f.=2, P=0.536) (27).

To our knowledge, the influence of ACE gene polymorphism on asthma has previously only been studied by Benessiano et al. (13). They examined 79 patients with asthma, 54 healthy subjects and 33 patients with non-asthmatic lung disease, and described a higher prevalence of the DD genotype in the first group. In contrast, we observed no significant difference in the genotype distributions between asthmatic patients and healthy controls. This might be related to the fact that they examined fewer healthy subjects, and added patients with non-asthmatic lung disease to the control population. Second, a racial difference may exist. Some investigators have indicated that the I/D ratio depends upon race. In Caucasians, the
I/D ratio was reported to be 0.41/0.59 by Rigat et al. (10), 0.44/0.55 by Lindpaintner et al. (19) and 0.43/0.57 by Benesistiano et al. (13), which means the D allele is dominant. However, in Japanese the I allele has been shown to be dominant by Furuya et al. (0.66/0.34) (25) and ourselves (0.65/0.34) (27). Third, the smaller difference in serum ACE levels among the three genotype in our asthmatic patients than that in healthy subjects might be of relevance. To our regret, since they did not describe serum ACE levels, we could not compare our data with their results. Finally, the fact that they did not correct for the mistyping that is said to occur for 4–5% of the DI type cases might also have affected their results (18,19,28).

No significant differences between patients and healthy controls were found regarding serum ACE levels in the present study, but since measurement was during periods of stability, this might not be totally informative. In future, we will concentrate attention on levels during asthma attack.

Although our results do not provide support for any association between the ACE gene polymorphism and asthma, they do not preclude a role for ACE in the pathogenesis of this disease. The potential association between ACE and asthma requires further investigation.

Acknowledgements

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References


