Bradykinin B₂ Receptor Gene Polymorphism Is Associated With Angiotensin-Converting Enzyme Inhibitor–Related Cough

Shuji Mukae, Shuichi Aoki, Seiji Itoh, Toshiki Iwata, Hiroaki Ueda, Takashi Katagiri

Abstract—The appearance of cough in association with angiotensin-converting enzyme (ACE) inhibitors is thought to be related to bradykinin, and it has been speculated that the elicitation of adverse effects is genetically predetermined. Several polymorphisms of the human bradykinin B₂ receptor gene may be involved in ACE inhibitor–related cough. To investigate this possibility, we identified the −58 thymine (T)/cytosine (C) polymorphism in subjects with ACE inhibitor–related cough. We classified the study population into 4 groups: subjects with and without cough that were treated with ACE inhibitors (n=30/30), nontreated essential hypertensive subjects (n=100), and normotensive subjects (n=100). The −58T/C was genotyped by the polymerase chain reaction single-strand conformation polymorphism method. The frequencies of the CC genotype and C allele of −58T/C were significantly higher in the nontreated hypertensive subjects than in the normotensive subjects. Conversely, the frequencies of the TT genotype and T allele were significantly higher in the subjects with cough than in the subjects without cough. These tendencies were more pronounced in females. Among the promoter assays of the human bradykinin B2 receptor, −58T was found to have a higher transcription rate than that of −58C. This finding seems to suggest that the transcriptional activity of promoter might be involved in the appearance of ACE inhibitor–related cough. A genetic variant of the bradykinin receptor is involved in the elicitation of ACE inhibitor–related cough. It may be possible to predict the side effects of ACE inhibitors in advance. (Hypertension. 2000;36:127-131.)

Key Words: bradykinin ■ genes ■ polymorphism ■ promoter

Angiotensin-converting enzyme (ACE) inhibitors have been widely used in therapy for hypertension, congestive heart failure, and myocardial infarction, and several large clinical trials have confirmed that ACE inhibitors reduce mortality and morbidity in patients with congestive heart failure.¹–⁵ Several studies also suggest that ACE inhibitors are efficient in left ventricular remodeling after acute myocardial infarction and congestive heart failure.⁶,⁷ The ability of ACE inhibitors to decrease angiotensin II production and increase kinin activity has been considered to be critically important. Reduced formation of angiotensin II seems to play a major role in the antihypertensive action of ACE inhibitors, but increased kinin levels have also been proposed to contribute to other beneficial effects of ACE inhibitors, including cardioprotection. However, ACE inhibitors also have adverse effects, the most common of which are cough and angioedema. ACE inhibitor–related cough is a side effect in ~10% of treated patients,⁸–¹⁰ and in some instances, an unexplained persistent cough limits the use of these drugs. Women are more likely to have this side effect, which may occur at any time from a few days to several months after the initiation of treatment. Why ACE inhibitors cause coughing is not completely understood. Accumulation of kinins has been suggested to play a major role in these adverse effects; it probably results from inhibition of the degradation of kinins, particularly bradykinin, in the airway, but the precise mechanism is still unknown. A genetic predisposition has been proposed on the basis of a similarity in the frequency of polymorphism in the gene for ACE and ACE inhibitor–related cough.¹¹ Speculations about a genetic predetermination of these adverse effects have specifically implicated variants of the genes encoding ACE, chymase, and bradykinin B₂ receptors.¹²

Bradykinin, a family of oligopeptides derived from the enzymatic action of kallikreins on kininogens, can promote all the major signs of inflammation, including hyperemia, leakage of plasma proteins, and pain.¹³–¹⁶ Kinins act mainly as local hormones by activating specific receptors, known as B₁ and B₂ receptors, with most of the inflammatory and cardiovascular effects being mediated by the B₂ receptor.¹⁷,¹⁸ Human bradykinin receptors are cell-surface G-protein–coupled receptors of the 7-transmembrane–domained superfamily.¹⁹ The human B₂ bradykinin receptor cDNA was recently cloned by Eggerickx et al,²⁰ Hess et al,²¹ and others,²²,²³ and
The cough symptoms disappeared soon after withdrawal of the ACE inhibitors. These patients had complained of dry cough, and all of their previous therapy with ACE inhibitors had to be withdrawn because of the development of a cough within 2 weeks after starting treatment of essential hypertension. They were all essential hypertensive subjects free of complications such as ischemic heart disease, hyperlipidemia, and diabetes mellitus. None had any history of vascular diseases.

To investigate the ACE inhibitor–related cough from the variants of the genes, we examined the distribution of a nucleotide polymorphism in the core promoter of the bradykinin B₂ receptor gene in Japanese subjects with a history of ACE inhibitor–related cough.

**Methods**

**Study Population**

We retrospectively studied the genetic susceptibility to ACE inhibitor–induced cough in hypertensive patients by examining the bradykinin B₂ receptor gene promoter polymorphism. The participants were randomly selected Japanese outpatients at Showa University Hospital and affiliated hospitals in Tokyo, Japan. We classified the study population into 4 groups. The first 2 groups included a combined total of 60 patients, with and without cough (cough+ and cough−, respectively), who had been given ACE inhibitors for the treatment of essential hypertension. They were all essential hypertensive subjects free of complications such as ischemic heart disease, hyperlipidemia, and diabetes mellitus. None had any history of recent respiratory infection, other respiratory diseases, or pulmonary congestion. In the cough+ patients (n=30, 11 male, aged 51±7 years), previous therapy with ACE inhibitors had to be withdrawn because of the development of a cough within 2 weeks after starting therapy. These patients had complained of dry cough, and all of their symptoms disappeared soon after withdrawal of the ACE inhibitors. The cough− patients (n=30, 11 male, aged 51±10 years), age- and gender-matched to the cough+ patients, had no complaints of cough and continued the ACE inhibitor regimen for the treatment of essential hypertension.

The third group included 100 nontreated subjects with essential hypertension who were also randomly selected outpatients at Showa University Hospital and affiliated hospitals; this group was used to investigate the genotypes and allelic frequencies of the bradykinin B₂ receptor gene. One hundred fourteen and 147 subjects with ACE-I 30 (11/19) 51

**Table 1. Characteristics of Normotensive, Nontreated Hypertensive, Cough+, and Cough− Subjects**

<table>
<thead>
<tr>
<th></th>
<th>n (Male/Female)</th>
<th>Age, y</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive subjects</td>
<td>100 (68/32)</td>
<td>52±10</td>
<td>NS</td>
</tr>
<tr>
<td>Nontreated hypertensive subjects</td>
<td>100 (70/30)</td>
<td>54±8</td>
<td>NS</td>
</tr>
<tr>
<td>Cough+ subjects with ACE-I</td>
<td>30 (11/19)</td>
<td>51±7</td>
<td>NS</td>
</tr>
<tr>
<td>Cough− subjects with ACE-I</td>
<td>30 (11/19)</td>
<td>51±10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Age is expressed as mean±SD, and comparison was performed by ANOVA. ACE-I indicates ACE inhibitor.

**Amplification of Promoter Polymorphism of the Human Bradykinin B₂ Receptor Gene**

The DNA of the subjects was extracted from leukocytes by use of the QIAamp kit (Qiagen). The primers for polymerase chain reaction (PCR) amplification were F (5-GCAGAGCTCGCTGAGGAG-3), located in the promoter, and R (5-CCTCCTGGAGCCAGAAG-3), located in the promoter/exon1. Primers were designed from the bradykinin B₂ receptor gene reported by Kammerer et al. The total reaction volume was 100 μL in a mixture containing 1 μg of genomic DNA, 50 ng of each primer, 200 μmol/L of each dNTP, 1.5 mmol/L of MgCl₂, and 0.5 U of Taq DNA polymerase. Cycle conditions for PCR were initially 5 minutes at 94°C, followed by 1 minute at 94°C, 30 seconds at 58°C, and 30 seconds at 72°C for 30 cycles, with a final extension time of 5 minutes at 72°C.

**Detection of Promoter Polymorphism**

PCR products were subjected to single-strand conformation polymorphism (SSCP) electrophoresis. A 10 μL aliquot of the PCR product was diluted with 30 μL formamide, denatured at 95°C for 10 minutes, and subjected to SSCP analysis in a 20% polyacrylamide (2× TBE) gel. Electrophoresis was carried out in 2× TBE buffer at 24°C at 180 V for 20 hours, and the gels were then silver-stained. SSCP analysis of 260 unrelated Japanese subjects was performed in the same way. Several samples representative of each genotype detected by SSCP were sequenced by fluorescent cycle sequencing to confirm the thymine (T) or cytosine (C) at nucleotide position –58 upstream from the putative transcription start site.

**Statistical Analysis**

The significance of differences in classified values among each subject was examined by χ² analysis, and the Fisher test was used for sets with small numbers. A value of P<0.05 was considered to indicate statistical significance.

**Results**

The backgrounds of normotensive subjects, nontreated hypertensive subjects, and cough+− subjects with ACE inhibitors are shown in Table 1.

![Figure 1](http://hyper.ahajournals.org/DownloadedFrom/10.1161/01.HYP.38.4.587)
are shown in Table 1. A promoter-specific 112-bp fragment was amplified by using the forward and reverse primers (Figure 1). In samples obtained from 260 unrelated Japanese individuals, 3 genotypes were disclosed by SSCP electrophoresis in a 20% polyacrylamide gel. DNA sequencing showed a T or C at nucleotide position −58 upstream from the putative transcription start site (Figure 2).

The distributions of the T/C genotypes and allelic frequencies of the bradykinin B2 receptor gene polymorphism in normotensive subjects, nontreated hypertensive subjects, and cough+/- subjects with ACE inhibitors are shown in Tables 2 and 3. The genotypes and allelic frequencies were in Hardy-Weinberg equilibrium. The distributions of the T/C genotypes were 18% for CC, 57% for TC, and 25% for TT in normotensive subjects and 28% for CC, 59% for TC, and 13% for TT in nontreated hypertensive subjects. A significantly higher incidence of the CC genotype was seen in the nontreated hypertensive subjects (χ²=5.998, P=0.049). Therefore, in the general hypertensive population (disregarding the presence or absence of cough), a significantly higher incidence of the CC genotype was seen in the hypertensive subjects than in the normotensive subjects. In contrast, the distributions of the T/C genotypes in cough+ subjects were 3% for CC, 60% for TC, and 37% for TT, and this was significantly different from the distributions in the cough− subjects (χ²=11.963, P=0.002) and nontreated hypertensive subjects (χ²=13.299, P=0.001). These tendencies were most apparent in the females. In the female cough+ subjects, the distributions of the T/C genotypes were 0% for CC, 53% for TC, and 47% for TT, and this was significantly different from the distributions in the female cough− subjects (χ²=11.454, P=0.003) and nontreated female hypertensive subjects (χ²=11.413, P=0.003).

The allelic frequencies were 0.465 for the C allele and 0.535 for the T allele in the normotensive subjects and 0.575 and 0.425, respectively, in the nontreated hypertensive subjects. A significant increase of the C allele was seen in the nontreated hypertensive subjects (χ²=4.847, P=0.027). Therefore, in the general hypertensive population (disregarding the presence or absence of cough), a significantly higher incidence of the C allele was seen in the hypertensive subjects than in the normotensive subjects. In contrast, in cough+ subjects, the allelic frequencies were 0.333 for the C allele and 0.667 for the T allele, and the frequency of the T allele was 0.003.

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TABLE 3. Allelic Frequencies of −58T/C for Human Bradykinin B2 Receptor Gene in Normotensive, Nontreated Hypertensive, Cough+, and Cough− Subjects

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>T</th>
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</thead>
<tbody>
<tr>
<td>Normotensive subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+F (n=100)</td>
<td>0.465</td>
<td>0.535</td>
</tr>
<tr>
<td>M (n=68)</td>
<td>0.449</td>
<td>0.551</td>
</tr>
<tr>
<td>F (n=32)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Nontreated hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+F (n=100)</td>
<td>0.575</td>
<td>0.425</td>
</tr>
<tr>
<td>M (n=70)</td>
<td>0.564</td>
<td>0.436</td>
</tr>
<tr>
<td>F (n=30)</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Cough+ with ACE-I subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+F (n=30)</td>
<td>0.333</td>
<td>0.667</td>
</tr>
<tr>
<td>M (n=11)</td>
<td>0.455</td>
<td>0.545</td>
</tr>
<tr>
<td>F (n=19)</td>
<td>0.263</td>
<td>0.737</td>
</tr>
<tr>
<td>Cough− with ACE-I subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+F (n=30)</td>
<td>0.617</td>
<td>0.383</td>
</tr>
<tr>
<td>M (n=11)</td>
<td>0.591</td>
<td>0.409</td>
</tr>
<tr>
<td>F (n=19)</td>
<td>0.632</td>
<td>0.368</td>
</tr>
</tbody>
</table>

Comparison was performed by χ² test or Fisher test. Test values were as follows: for normotensive, nontreated hypertensive, cough+, and cough− subjects, χ²=15.226 and P=0.001 (M+F); χ²=4.553 and P=0.027 (M); and χ²=13.483 and P=0.003 (F); for normotensive subjects vs nontreated hypertensive subjects, χ²=4.847 and P=0.027 (M+F); χ²=3.698 and P=0.054 (M); and χ²=1.250 and P=0.263 (F); for cough+ subjects vs cough− subjects, χ²=0.657 and P=0.001 (M+F); χ²=0.819 and P=0.365 (M); and χ²=10.431 and P=0.001 (F); and for cough+ subjects vs nontreated hypertensive subjects, χ²=10.798 and P=0.001 (M+F); χ²=0.924 and P=0.336 (M), and χ²=10.598 and P=0.001 (F).

was significantly higher in these subjects than in the cough− subjects (χ²=9.657, P=0.001) and in the nontreated hypertensive subjects (χ²=10.798, P=0.001). Just as in the case of the distributions of the T/C genotypes, a significant increase of the T allele was seen in the female cough+ subjects versus the cough− subjects (χ²=10.431, P=0.001) and versus the nontreated hypertensive subjects (χ²=10.598, P=0.001).

Subjects with the TT genotype were most susceptible to cough, and subjects with CC genotype were least susceptible, especially among females. There was evidence of an association between genotype and cough.

Discussion
In recent years, the clinical use of ACE inhibitors has been increasingly applied for the treatment of hypertension, congestive heart failure, and myocardial infarction. The mechanism of the benefit for left ventricular systolic dysfunction has been thought to be largely due to the prevention of left ventricular dilatation and remodeling.6,7 The drawback, however, is that ACE inhibitors have been reported to cause dry cough as a side effect in 1% to 33% of the patients.8−10 Dry cough is the most common and unexplained symptom, and although it can often be annoying, it seldom is harmful. Persistent cough, usually more severe at night, forces a significant number of patients to discontinue use of the drugs. The cough may improve with a reduction in the dose but is usually not wholly dose dependent.27

After withdrawal, it abates within 1 days to 4 weeks. The symptom seems to be more prevalent in females than in males; in most larger studies, two thirds of the affected patients are females.28 The symptom is also more common in nonsmokers than in smokers.

Despite considerable scientific investigation on the cause and mechanism of the dry cough induced by ACE inhibition, the specific mechanism of this adverse effect is not fully understood. It may be related to effects on the kininogen-kinin (bradykinin) system because the breakdown of bradykinin is known to be prevented by ACE inhibitors. The accumulation of kinins has been suggested to play a major role in these adverse effects of ACE inhibitors. The appearance of a cough has been attributed to a possible local accumulation of bradykinin. A local accumulation of bradykinin may lead to activation of a proinflammatory peptide (eg, substance P and neuropeptide Y) and a local release of histamine, and those, in turn, may additionally induce cough reflex hypersensitivity. For these reasons, most research on putative mechanisms has focused on the effects mediated by bradykinin.29

Prostaglandins have also been implicated in ACE inhibitor–related cough because both bradykinin and substance P act via common second messengers, some of which are the prostaglandins.30−32 It has been proposed that bradykinin increases lung prostaglandin E₂ levels, which produce cough and increase bronchial reactivity by stimulating unmethylated C-afferent fibers.15,33,34 ACE inhibitors may generate bronchoactive mediators, such as prostaglandins,15 and then may decrease the bronchodilator effects of vasoactive intestinal peptide or β-agonist by preventing the accumulation of cAMP in smooth muscle.35

On the other hand, bradykinin plays an important role in the cardiovascular system, affecting blood pressure regulation, cell proliferation, and matrix synthesis by fibroblasts.15,18 By coupling to G proteins, the bradykinin B₂ receptor triggers the activation of phospholipase C and/or phospholipase A₂ that accompanies increased intracellular levels of Ca²⁺, NO, cGMP, and/or cAMP.19 The human B₂ receptor gene has been cloned20−23 and mapped to human chromosome region 14q32.24 The gene is larger than 25 kb and consists of 3 exons. In recent investigative studies involving the transgenic mouse model, the role of the bradykinin B₂ receptor in blood pressure regulation has also been substantiated by blood pressure reductions in transgenic mice that overexpress human bradykinin B₂ receptors.37 The results of these studies can be taken as reliable confirmation that the human B₂ receptor genes are involved in hypertension.

Moreover, it has been speculated that the occurrence of adverse effects of ACE inhibitors is genetically predetermined. The candidate genes implicated thus far include variants of the genes encoding the ACE gene,11 chymase, and bradykinin B₂ receptors.12 To determine whether genetic variants in the bradykinin B₂ receptor gene could affect receptor expression and function and induce ACE inhibitor–related cough, we retrospectively studied the genetic susceptibility to ACE inhibitor–related cough in patients with hypertension by examining bradykinin B₂ receptor gene polymorphism.

On the basis of a determination of genotypes for promoter polymorphism for the bradykinin B₂ receptor gene in subjects...
with a history of ACE inhibitor–related cough, we found significant associations between the TT genotype, the T allele of bradykinin B2 receptor gene promoter polymorphism, and ACE inhibitor–related cough. These associations were more apparent in females. Subjects with the TT genotype were most susceptible to cough, and subjects with the CC genotype were least susceptible, especially among females. These data show the association between bradykinin B2 receptor gene polymorphism and susceptibility to ACE inhibitor–induced cough.

After finding that the polymorphism of the promoter region may influence the transcription rate of the bradykinin B2 receptor gene, Braun et al.\(^{26}\) initiated in vitro transfection experiments in human embryonic kidney cells and performed luciferase reporter gene assays to examine how the transcription rate is affected by the different alleles of the promoter. A reduction of the transcriptional activity was obtained by combining the promoter region with exon 1, and the luciferase reporter gene assay of \(-58T\) was found to be higher than that of \(-58C\). Kammerer et al.\(^{25}\) reported that the promoter and exon 1 of the bradykinin B2 receptor are also related to the transcription rate. According to our results, the transcriptional activity of the bradykinin B2 receptor promoter might be involved in the occurrence of ACE inhibitor–related cough, and high transcriptional activity of the bradykinin B2 receptor promoter might induce ACE inhibitor–related cough.

In the present study, we retrospectively investigated the genetic susceptibility to ACE inhibitor–related cough by examining bradykinin B2 receptor gene promoter polymorphism. As a result, we found that a genetic variation of the gene may explain the occurrence of this adverse drug reaction. This genetic variation might be an effective predictor of ACE inhibitor–related cough in advance.

### References

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