Association of Asthma Severity and Bronchial Hyperresponsiveness With a Polymorphism in the Cytotoxic T-Lymphocyte Antigen-4 Gene*

Sang Yeub Lee, MD; Young Ho Lee, MD; Chol Shin, MD; Jae Jeong Shim, MD; Kyung Ho Kang, MD, FCCP; Se Hwa Yoo, MD; and Kwang Ho In, MD

**Objectives:** Cytotoxic T-lymphocyte antigen (CTLA)-4 is a homolog of CD28, which is expressed only on activated T cells. It binds to accessory molecule B7 and mediates T-cell-dependent immune response. Signaling through CTLA-4 may down-regulate type 1 T-helper cell proliferation; moreover, some studies suggest that CTLA-4 might also deliver a positive signal to type 2 T-helper cell activation. Disruption of this delicate balance of immune regulation may lead to autoimmune diseases or atopic diseases. To evaluate the possible role of CTLA-4 polymorphisms in bronchial asthma, we investigated the association between polymorphisms (exon 1 +49 A/G, promoter −318 C/T) and atopy, asthma severity, and bronchial hyperresponsiveness in bronchial asthma patients and a group of healthy control subjects.

**Patients:** Eighty-eight asthmatic patients and 88 healthy control subjects were studied.

**Measurements and results:** Asthma severity assessment, methacholine challenge, allergy skin-prick test, and serum total IgE measurements were performed. The genotypes of the CTLA-4 promoter (−318 C/T) and exon 1 (+49 A/G) in all subjects were determined using the polymerase chain reaction and restriction fragment length polymorphism. The CTLA-4 promoter (−318 C/T) polymorphism was shown to be associated with asthma severity, but not with asthma, atopy, or bronchial hyperresponsiveness. A significant association was found between severe asthma and the T allele (p = 0.037). The CTLA-4 exon 1 (+49 A/G) polymorphism was shown to be associated with bronchial hyperresponsiveness, but not with asthma, atopy, or asthma severity. Asthmatic patients of the GG genotype had more hyperresponsive airways than those with the AG or AA genotype (p = 0.019).

**Conclusions:** The CTLA-4 promoter (−318 C/T) T allele may serve as a clinically useful marker of severe asthma. The CTLA-4 exon 1 (+49 A/G) polymorphism may have a disease-modifying effect in asthmatic airways.

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**Key words:** asthma; bronchial hyperresponsiveness; cytotoxic T-lymphocyte antigen-4 polymorphism

**Abbreviations:** APC = antigen-presenting cell; bp = base pair; CTLA = cytotoxic T-lymphocyte antigen; IL = interleukin; PC20 = provocative concentration of methacholine causing a 20% fall in FEV1; PCR = polymerase chain reaction; PEF = peak expiratory flow; RFLP = restriction fragment length polymorphism; Th1 = type 1 T-helper; Th2 = type 2 T-helper

Bronchial asthma is characterized by the infiltration of inflammatory cells into the airway submucosa. While the precise mechanisms by which inflammatory cells are recruited into the lungs are not fully understood, increasingly available evidence suggests that the activation of antigen-specific CD4+ T cells of the type 2 T-helper (Th2) subset in the lungs, which results in interleukin (IL)-5 secretion plays a major role in asthmatic airway inflammation. CD4+ T-cell activation leading to cytokine production and effector function requires two signals from the antigen-presenting cell (APC). The first signal is triggered by the interaction between antigen-specific T-cell receptor and peptide-major histocompatibility complex II complexes on APCs. The second signal or "costimulatory" signal is triggered by CD80 (B7–1) and CD86 (B7–2) of the APC binding...
to the CD28 and cytotoxic T-lymphocyte antigen (CTLA)-4 of the T lymphocyte. In the absence of costimulatory signals, the T-cell-dependent immune response is greatly diminished, or even eliminated, and costimulatory signals may, therefore, fulfill a valuable role in T-lymphocyte activation, type 1 T-helper (Th1) or Th2 cell differentiation, and the production of various cytokines.

CTLA-4 is a second costimulatory molecule and is a homolog of CD28. It is expressed only on activated T cells, binds to accessory molecule B7, and mediates T-cell-dependent immune response. Signaling through CTLA-4 may down-regulate Th1 cell proliferation by inhibiting the production of IL-2 and IL-2 receptor expression. However, the role of CTLA-4 remains uncertain, with some studies suggesting that CTLA-4 might also deliver a positive signal to Th2 cell activation. Disruption of this delicate balance of immune regulation could lead to autoimmune diseases or atopic diseases. Therefore, CTLA-4 is considered to be important in the development of many of the immunologic and physiologic features of asthma.

Polymorphisms of the CTLA-4 gene, located on chromosome 2q33, could thus have effects on immune response. The following three CTLA-4 gene polymorphisms are known: a microsatellite (AT)n marker at position 642 of the 3’-untranslated region of exon 4; a single-nucleotide polymorphism in exon 1 (adenine or guanine at position 49); and another single-nucleotide polymorphism in the promoter region (cytosine or thymine at position −318). The A/G dimorphism at exon 1 position +49 results in an amino acid exchange (threonine to alanine) in the leader sequence.

To evaluate the possible role of CTLA-4 polymorphisms in bronchial asthma, we investigated the association between two polymorphisms (exon 1 +49 A/G, promoter −318 C/T) and atopy, asthma severity, and bronchial hyperresponsiveness, in bronchial asthma patients and a healthy control group.

Materials and Methods

Subjects

Eighty-eight consecutive patients with asthma (35 male and 53 female patients), whose ages ranged from 16 to 67 years (mean, 44.2 years), were prospectively studied. The subjects were considered asthmatic if they had a prior clinical history of asthma (as defined by the American Thoracic Society) for ≥ 1 year and had, within the year preceding the study, a history of at least one of the following: (1) a > 12% reversibility of FEV₁ in response to a bronchodilator; (2) a result of a challenge with a provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀) of < 8 mg/mL; or (3) a > 20% maximum within-day amplitude from twice the daily peak expiratory flow (PEF) measurement over a 2-week period. All patients were nonsmokers or ex-smokers (for > 12 months, with < 5 pack-years).

Eighty-six healthy subjects (19 men and 67 women), whose ages ranged from 22 to 68 years (mean, 46.0 years), were recruited from the employee pool of the Korea University Hospital and graduate students at the College of Medicine, Korea University, and served as control subjects. All control subjects were nonsmokers and had normal findings on chest radiographs, normal airway reactivity, and normal pulmonary function test results. Moreover, they had experienced no current respiratory symptoms, were nonatopic, and had experienced no respiratory infections within the previous month. None were receiving any medications. The study protocol was approved by the ethics committee of the Korea University College of Medicine and written informed consent was obtained from all subjects.

Clinical Assessment

The methacholine challenge was performed using the following method. Acetyl methacholine chloride (Sigma; St. Louis, MO) was diluted with normal saline solution and divided into nine solutions ranging from 0.075 to 25 mg/mL. Each solution was inhaled five times through a nebulizer (DeVilbiss Pulmoaide Compressor/Nebulizer; SM Instruments; Doylestown, PA) and a

**Figure 1.** CTLA-4 exon 1 and promoter genotypes by PCR-RFLP. The GG (152 bp), AG (152 bp, 130 bp), AA (130 bp), CC (226 bp), CT (226 bp, 130 bp, 96 bp), and TT (130 bp, 96 bp) genotypes are shown.
Healthy control subjects

prick test or a total serum IgE level of then 30 cycles of 40 s at 94°.

conditions were applied: initial denaturation for 4 min at 94°.

A 247-base pair (bp) fragment was amplified by PCR. The following

that there was no reaction induced by the allergen but that there

solution were measured with a skin test reaction gauge (Bencard)

Brentford, England) of 55 common allergen extracts. The ratios

to a wheal size ratio of /H11021

scores

/H11022

FEV1 was measured after each inhalation, and the PC20 was

Dosimeter (Micro-Dosimeter; Sunrise Medical; Carlsbad, CA).

Atopy was defined as a positive reaction to the allergy skin-

prick test or a total serum IgE level of > 100 IU/mL. The allergy

skin-prick tests were performed using a solution (Bencard;

Brentford, England) of 55 common allergen extracts. The ratios

of the wheal size compared with that induced by a histamine

solution were measured with a skin test reaction gauge (Bencard)

15 min after skin pricking. A negative result of the test indicated

that there was no reaction induced by the allergen but that there

was a normal reaction to the controlled histamine solution. A

score of 1+ was given to erythema, and a score of 2+ was given to

a wheal size ratio of < 1. Ratios between 1 and 2 were given as a

score of 3+, and ratios between 2 and 3 were given as a 4+. Scores

> 2+ were designated as positive. Total serum IgE levels

were measured with paper radioimmunoassort test kits (Radim;

Rome, Italy), and levels > 100 IU/mL were defined as positive.

Classification of asthma severity was based on history, symp-

toms, clinical features, medication need, FEV1, and PEF accord-

ing to the 1997 Expert Panel Report 2.15 Asthma severity was

classified into mild intermittent, mild persistent, moderate per-

sistent, and severe persistent. The investigator who assessed the

severity of asthma was blinded to the genotyping of the CTLA-4

gene.

Genotyping of CTLA-4 Gene

DNA was extracted from peripheral blood leukocytes following

standard protocols and column purified (DNA midi kit; Qiagen;

Hilden, Germany). The CTLA-4 promoter polymorphism at position

−318 was defined using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) with

Tru9I restriction enzymes (Bioneer; Seoul, Korea). To amplify

the target DNA in the CTLA-4 promoter, PCR was performed

with oligonucleotides forward 5'-AAAGCTCAGCTGAACCTGGT-3'

and reverse 5'-CTGAAACAAATGAAACCC-3'.13 A

247-base pair (bp) fragment was amplified by PCR. The following

conditions were applied: initial denaturation for 4 min at 94°C,

then 30 cycles of 40 s at 94°C, 15 min after skin pricking. A negative result of the test indicated that there was no reaction induced by the allergen but that there was a normal reaction to the controlled histamine solution. A score of 1+ was given to erythema, and a score of 2+ was given to a wheal size ratio of < 1. Ratios between 1 and 2 were given as a score of 3+, and ratios between 2 and 3 were given as a 4+. Scores > 2+ were designated as positive. Total serum IgE levels were measured with paper radioimmunoassort test kits (Radim; Rome, Italy), and levels > 100 IU/mL were defined as positive.

Classification of asthma severity was based on history, symptoms, clinical features, medication need, FEV1, and PEF according to the 1997 Expert Panel Report 2.15 Asthma severity was classified into mild intermittent, mild persistent, moderate persistent, and severe persistent. The investigator who assessed the severity of asthma was blinded to the genotyping of the CTLA-4 gene.

Genotyping of CTLA-4 Gene

DNA was extracted from peripheral blood leukocytes following standard protocols and column purified (DNA midi kit; Qiagen; Hilden, Germany). The CTLA-4 promoter polymorphism at position −318 was defined using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) with Tru9I restriction enzymes (Bioneer; Seoul, Korea). To amplify the target DNA in the CTLA-4 promoter, PCR was performed with oligonucleotides forward 5'-AAAGCTCAGCTGAACCTGGT-3' and reverse 5'-CTGAAACAAATGAAACCC-3'.13 A 247-base pair (bp) fragment was amplified by PCR. The following conditions were applied: initial denaturation for 4 min at 94°C, then 30 cycles of 40 s at 94°C, 40 s at 60°C, and 30 s at 72°C. This was followed by a final extension for 4 min at 72°C in a thermal cycler (model 9600; PerkinElmer; Boston, MA). PCR products were further subjected to RFLP analysis with the enzyme Tru9I (Bioneer) and separated on a 3% agarose gel. PCR fragments with thymine at position −318 were cut into three fragments (21, 96, and 130 bp), whereas fragments with cytosine at the same position only had the restriction site at 21 bp. The CTLA-4 exon 1 position 49 (codon 17) polymorphism was defined using PCR-RFLP with the BstEII restriction enzyme (Promega; Madison, WI). PCR was carried out using forward primer 5'-AAGGCTCAGCTGAACCTGGT-3' and reverse primer 5'-CTGAAACAAATGAAACCC-3'.16 Samples were denatured by 30 cycles of 30 s at 94°C, annealed for 30 s at 58°C, and extended for 30 s at 72°C, using the thermal cycler. A 152-bp fragment containing the +49 A/G polymorphism in exon 1 of CTLA-4 was amplified. The forward primer was designed with a single-base mismatch for the last nucleotide, which corresponded to the +47 position, to introduce a base change in the sequence of the PCR product. This substitution created a BstEII restriction site in the A allele. Amplified products were incubated at 60°C for 2 h using 5 U BstEII per reaction. Digested products underwent electrophoresis on a 3.5% agarose gel. The digested A allele gave a fragment of 130 bp, and the G allele gave an intact 152-bp fragment (Fig 1).

Statistical Analysis

Statistical analysis was performed using statistical software (SPSS, version 8.0; SPSS; Chicago, IL). The χ² test and t test were used when appropriate.

Results

CTLA-4 Promoter (−318 C/T) Polymorphism

No deviation from the Hardy-Weinberg equilibrium was observed in any group examined (asthma

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Table 1—CTLA-4 Promoter (−318 C/T) Polymorphism in Asthmatic Patients and Healthy Control Subjects*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>Asthma patients (n = 88)</td>
<td>70 (79.5)</td>
</tr>
<tr>
<td>Healthy control subjects (n = 86)</td>
<td>67 (77.9)</td>
</tr>
</tbody>
</table>

*Values given as No. (%). p = 0.690 (χ²).

Table 2—CTLA-4 Promoter (−318 C/T) Polymorphism According to Atopy*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>Atopic asthma patients (n = 52)</td>
<td>38 (73.1)</td>
</tr>
<tr>
<td>Nonatopic asthma patients (n = 36)</td>
<td>32 (88.9)</td>
</tr>
<tr>
<td>Healthy control subjects (n = 86)</td>
<td>67 (77.9)</td>
</tr>
</tbody>
</table>

*Values given as No. (%). p = 0.311 (χ²).

Table 3—CTLA-4 Promoter (−318 C/T) Polymorphism According to Severity of Asthma*

<table>
<thead>
<tr>
<th>Asthma Severity</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>Mild to moderate (n = 61)</td>
<td>53 (86.9)</td>
</tr>
<tr>
<td>Severe (n = 27)</td>
<td>17 (63.0)</td>
</tr>
<tr>
<td>C Allele</td>
<td>113 (92.6)</td>
</tr>
<tr>
<td>T Allele</td>
<td></td>
</tr>
</tbody>
</table>

*Values given as No. (%). p = 0.037 (χ²).

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group, $\chi^2 = 0.458$ and $p = 0.795$; control group, $\chi^2 = 1.168$ and $p = 0.558$). The distribution of genotypic frequencies was not significantly different for the asthma group and the control group ($p = 0.690$; Table 1). When the asthma group was divided into patients with atopic asthma and nonatopic asthma, the distribution of genotypic frequencies did not differ among the atopic asthma group, the nonatopic asthma group, and the control group ($p = 0.311$; Table 2). The relationships between the CTLA-4 promoter (-318 C/T) polymorphism and asthma severity were studied. Sixty-one patients were classified as having mild-to-moderate asthma (mild intermittent, 17 patients; mild persistent, 31 patients; moderate persistent, 13 patients), and 27 patients had severe asthma. The distributions of genotypic frequencies were significantly different for the mild-to-moderate group and the severe group. In particular, the frequency of the T allele was 7.4% in the mild-to-moderate group and 20.4% in the severe group, and the association between severe asthma and the T allele was significant ($p = 0.037$; Table 3). The positive predictive value of a positive T allele for severe asthma was 55.0% and the negative predictive value was 72.4%. The relationship between bronchial hyperresponsiveness (PC$_{20}$) and the CTLA-4 exon 1 (+49 A/G) polymorphism was significant ($p = 0.019$; Table 8). PC$_{20}$ was measured in 45 of the 88 asthma patients, classified into a GG genotype (wild-type) group and an AG genotype +AA genotype (mutant-type) group. The geometric mean (± SD) PC$_{20}$ in the wild-type group was $2.19 \pm 2.12$ mg/mL, and in the mutant-type group was $4.51 \pm 3.19$ mg/mL. From these results, asthmatic patients with the GG genotype had more hyperresponsive airways than did those with the AG or AA genotypes.

**Discussion**

In this study, the CTLA-4 promoter (-318 C/T) polymorphism was found to be associated with asthma severity, but not with asthma, atopy, or bronchial hyperresponsiveness. The CTLA-4 exon 1 (+49 A/G) polymorphism was shown to be associated with bronchial hyperresponsiveness, but not with asthma, atopy, or asthma severity. From these results, it appears that the CTLA-4 gene may not be a susceptibility gene, but rather a disease-modifying gene that can modify the asthma phenotype.

There is also increasing evidence that CTLA-4 polymorphisms confer susceptibility to several autoimmune disorders. In particular, the exon 1 (+49 A/G) polymorphism has been found to be associated with type 1 diabetes, Graves disease, DR4-positive rheumatoid arthritis, multiple sclerosis,

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No.</th>
<th>PC$_{20}$, mg/mL$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>37</td>
<td>3.02 ± 2.75</td>
</tr>
<tr>
<td>CT + TT</td>
<td>8</td>
<td>2.68 ± 2.81</td>
</tr>
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</table>

$^*$Values given as geometric mean ± SD. $p = 0.758$ (t test).

**CTLA-4 Exon 1 (+49 A/G) Polymorphism**

No deviation from the Hardy-Weinberg equilibrium was observed in any group examined (asthma group, $\chi^2 = 3.938$ and $p = 0.140$; control group, $\chi^2 = 0.585$ and $p = 0.746$). The distribution of genotypic frequencies was not different for the asthma group and the control group ($p = 0.275$; Table 5), nor for the atopic asthma group, the nonatopic asthma group, and the control group ($p = 0.563$; Table 6). In addition, the mild-to-moderate asthma group and the severe asthma group did not differ in the distribution of genotype frequencies ($p = 0.166$; Table 7). However, the association between bronchial hyperresponsiveness (PC$_{20}$) and the CTLA-4 exon 1 (+49 A/G) polymorphism was significant ($p = 0.037$; Table 3). PC$_{20}$ was measured in 45 of the 88 asthma patients, classified into a GG genotype (wild-type) group and an AG genotype +AA genotype (mutant-type) group. The geometric mean (± SD) PC$_{20}$ in the wild-type group was $2.19 \pm 2.12$ mg/mL, and in the mutant-type group was $4.51 \pm 3.19$ mg/mL. From these results, asthmatic patients with the GG genotype had more hyperresponsive airways than did those with the AG or AA genotypes.

**Table 6—CTLA-4 Exon 1 (+49 A/G) Polymorphism According to atopy**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No.</th>
<th>GG</th>
<th>AG</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic asthma patients (n = 52)</td>
<td>28 (53.8)</td>
<td>14 (26.9)</td>
<td>10 (19.2)</td>
<td></td>
</tr>
<tr>
<td>Nonatopic asthma patients (n = 36)</td>
<td>21 (58.3)</td>
<td>10 (27.7)</td>
<td>5 (13.9)</td>
<td></td>
</tr>
<tr>
<td>Healthy control subjects (n = 86)</td>
<td>49 (57.0)</td>
<td>29 (33.7)</td>
<td>8 (9.3)</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Values given as No. (%). $p = 0.563$ ($\chi^2$).
and celiac disease. The exon 1 (+49 A/G) GG genotype or the G allele has been found to be associated with type 1 diabetes, Graves diseases, and DR4-positive rheumatoid arthritis. Similarly, the AG genotype has been associated with multiple sclerosis, and the A allele has been associated with celiac disease. In this study, the CTLA-4 exon 1 (+49 A/G) polymorphism was associated with bronchial hyperresponsiveness. Asthmatic patients with the GG genotype had more hyperresponsive airways than did those with the AG or AA genotypes. The manner in which the GG genotype influences bronchial hyperresponsiveness is unclear. In the GG genotype patients, there probably is an increase in CTLA-4 activation. In an ovalbumin-sensitive murine model of asthma, treatment with CTLA-4-Ig significantly reduced the generation of IL-4, IL-5, and IgE, and reduced eosinophilic inflammation in the lungs, as well as reducing bronchial hyperresponsiveness in response to inhaled allergen. In the bronchial explant model, bronchial tissues from atopic asthmatics sensitive to the house dust mite were cultured for 24 h in the presence of house dust mite allergen extract. When CTLA-4-Ig was treated, the expression of IL-5, IL-13, IL-16, and RANTES (regulated on activation, normal T-cell expression and secretion) were reduced, as was T-cell chemotactic activity. Therefore, we can hypothesize that in GG genotype patients, the CTLA-4 is up-regulated, and that this could lead to increased T-cell chemotactic activity and ultimately could result in increased bronchial hyperresponsiveness. Functional studies will be necessary to establish intracellular CTLA-4 expression.

The CTLA-4 promoter (−318 C/T) polymorphism was associated with asthma severity. The frequency of the T allele was 7.4% in the mild-to-moderate group and 20.4% in the severe group, and this association between severe asthma and the T allele was significant (p = 0.037). Asthma severity was determined according to the 1997 Expert Panel Report 2, which included various clinical parameters, such as the degree and frequency of symptoms, the frequency of exacerbation, FEV1, and PEF. Therefore, it is difficult to explain how the promoter (−318 C/T) T allele affects asthma severity. However, it may be useful as a genetic marker of severe asthma. Early recognition of infants at risk for severe asthma by determination of their CTLA-4 promoter (−318 C/T) T allele followed by close medical follow-up and early environmental or pharmacologic intervention may delay, attenuate, or prevent the progression of disease. In adults identified as “at risk” for severe asthma at the time of diagnosis, closer medical follow-up and early institution of therapy may alter their outcomes.

The first possible shortcoming of this study was that the methacholine challenge was performed in only 45 of 88 patients, which would have resulted in a selection bias. The most important reason for not performing the methacholine challenge concerned the patients’ respiratory state. It was difficult to perform the test when FEV1/FVC was reduced or when symptoms such as cough, dyspnea, and wheezing were present. Another reason was patient refusal. A much larger number of subjects would have to be studied in the future. Second, the male/female ratio differed significantly between the control group and the asthmatic group. Therefore, we analyzed the association between sex and the polymorphisms (exon 1 +49 A/G, promoter −318 C/T) in all 174 subjects (54 male subjects and 120 female subjects) in the asthmatic and control groups. For the CTLA-4 promoter (−318 C/T) polymorphism, the distribution of genotypic frequencies did not differ by sex in all subjects (χ2, p = 0.738), the asthmatic group (χ2, p = 0.943), or the control group (χ2, p = 0.516). For the CTLA-4 exon 1 (+49 A/G) polymorphism, the distribution of genotypic frequencies did not differ by sex in all subjects (χ2, p = 0.818), the asthmatic group (χ2, p = 0.191), or the control group (χ2, p = 0.914). Therefore, we

### Table 7—CTL A-4 Exon 1 (+49 A/G) Polymorphism According to Severity of Asthma

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No.</th>
<th>PC20, mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>30</td>
<td>2.19 ± 2.12</td>
</tr>
<tr>
<td>AG + AA</td>
<td>15</td>
<td>4.51 ± 3.19</td>
</tr>
</tbody>
</table>

*Values given as No. (percent), p = 0.016 (χ2).

### Table 8—Relationship Between CTLA-4 Exon 1 (+49 A/G) Polymorphism and PC20

<table>
<thead>
<tr>
<th>Genotypes</th>
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*Values given as geometric mean ± SD, p = 0.019 (t test).
think it is possible that the difference in male/female ratios did not influence the conclusion of the study.

In conclusion, the CTLA-4 promoter (−318 C/T) T allele may serve as a clinically useful marker of severe asthma, and the CTLA-4 exon 1 (+49 A/G) polymorphism may have a disease-modifying effect in asthmatic airways.

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