

TGFB1 promoter polymorphism C-509T and pathophysiology of asthma

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Background: TGF- β 1 can modulate airway inflammation and exaggerate airway remodeling. A polymorphism of a promoter region of *TGFB1*, C-509T, might be associated with the development of asthma, but its pathophysiologic relevance remains poorly understood.

Objective: We investigated relations of the C-509T polymorphism to airflow obstruction, sputum eosinophilia, and airway wall thickening, as assessed by means of computed tomography, in 85 patients with stable asthma.

Methods: The CC, CT, and TT genotypes were examined by means of PCR and restriction enzyme fragment length polymorphism. At a selected bronchus, 3 indices of airway wall thickness were measured with an automatic method.

Results: The CC, CT, and TT genotypes were found in 22, 46, and 17 patients, respectively. Serum TGF- β 1 levels were significantly associated with the polymorphism and were increased in the CT/TT genotypes. FEV₁ and sputum eosinophil percentages were also significantly associated with the polymorphism and were both decreased in the CT/TT genotypes. The polymorphism was unrelated to airway wall thickness.

Conclusion: In addition to increased serum TGF- β 1 levels, the T allele of the C-509T polymorphism is related to increased airflow obstruction but attenuated eosinophilic inflammation. The former relation is not attributed to thickening of the central airway walls. The latter relation might reflect the anti-inflammatory effect of TGF- β 1. The C-509T polymorphism has a complex role in asthma pathophysiology, presumably because of the diverse functions of TGF- β 1 and its various interactions with cells and humoral factors *in vivo*.

(J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, airway remodeling, airway inflammation, TGF- β 1, C-509T, gene polymorphism, computed tomography, airway wall thickening, sputum eosinophil

Asthma is a chronic inflammatory disorder of the airways characterized by airflow obstruction, airway hyperresponsiveness, and airway wall remodeling. The pathogenesis of remodeling involves goblet cell and submucosal gland hyperplasia, vascular proliferation, deposition of extracellular matrix in the subepithelium and submucosa, and hypertrophy/hyperplasia of airway smooth muscle, which can lead to thickening of the airway wall.¹ The combination of epithelial damage, prolonged epithelial repair, overproduction of profibrotic growth factors, and proliferation and differentiation of fibroblasts is considered central to remodeling.² Airway remodeling can result in chronic airflow obstruction and more severe disease.³

TGF- β 1 is an important fibrogenic and immunomodulatory factor considered to have pivotal roles in the pathogenesis of airway remodeling.⁴ TGF- β 1 promotes proliferation of fibroblasts and deposition of collagen.⁵ TGF- β 1 immunoreactivity and mRNA expression in the airway submucosa are higher in patients with asthma than in healthy control subjects and are associated with the severity of disease and subepithelial fibrosis.⁶⁻⁸ On the other hand, TGF- β 1 might also function as a downregulator of the immune response. Studies in rodent models have demonstrated anti-inflammatory effects of TGF- β 1.^{9,10}

Several polymorphisms have recently been identified in the *TGFB1* gene promoter.¹¹⁻¹³ Among them, a C-to-T base exchange at bp -509 from the first major transcription initiation site has been linked to promoter activity enhancement.¹⁴ The T allele of *TGFB1*, C-509T, is associated with increased serum levels of TGF- β 1^{15,16} and IgE¹¹ and is significantly more common in asthmatic patients than in control subjects.^{12,14,16} This polymorphism might¹² or might not¹⁴ be associated with asthma severity, but its relation to airway remodeling remains unknown. Pathologic evaluation of airway remodeling requires endobronchial biopsy, which is invasive and unsuitable for large studies.

Computed tomography (CT) has recently been used to indirectly yet noninvasively assess airway remodeling.¹⁷⁻¹⁹ Wall thickening of asthmatic airways has been shown to be related to disease severity and airflow obstruction.²⁰⁻²² In this study we examined the relations of C-509T polymorphisms to serum TGF- β 1 levels, pulmonary function, and airway inflammation, as evaluated on the basis of induced sputum, and airway wall thickness, as assessed by means of CT in patients with stable asthma.

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication March 29, 2007; revised October 4, 2007; accepted for publication October 8, 2007.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.10.005

Abbreviations used

CT: Computed tomography
WA: Airway wall area

METHODS**Subjects**

We studied 85 nonsmoking patients with stable asthma. Asthma was diagnosed according to American Thoracic Society criteria.²³ Atopy was determined on the basis of the presence of specific serum IgE antibodies to at least 1 common inhalant allergen. Patients were classified into 4 groups according to asthma severity (intermittent, mild persistent, moderate persistent, and severe persistent), as defined by the step classification of the Global Initiative for Asthma guidelines.²⁴ No patient had evidence of chronic obstructive pulmonary disease. The study protocol was approved by the ethics committee at our institution, and written informed consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from peripheral blood cells with the use of commercial kits (Qiagen, Hilden, Germany). The genotypes of C-509T of the *TGFB1* gene were determined by means of PCR-RFLP, as described previously.¹² PCR primers were 5'-GGGGACAGTAAATGTATTGG (sense) and 5'-TGAGACACAGGGGAGCC (antisense). The reaction mixture contained 1× PCR buffer, 1.5 mmol/L MgCl₂, 200 μmol/L deoxynucleotide triphosphates, 2.5 U of Taq DNA polymerase (Applied Biosystems, Foster City, Calif), 0.5 μmol/L of each primer, and 50 to 100 ng of genomic DNA. The PCR amplification reaction consisted of 1 cycle at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 61°C for 1 minute, and extension at 72°C for 1 minute. The PCR products were digested with restriction endonuclease *DdeI* (Promega, Madison, Wis) and electrophoresed on a 3% agarose gel stained with ethidium bromide. The T allele resulted in 288-bp and 115-bp fragments, whereas the C allele resulted in 235-bp and 115-bp fragments.

Sputum induction

After premedication with inhaled salbutamol, sputum was induced as described previously.^{25,26} The percentage of eosinophils was determined on centrifuged preparations stained with May-Grunwald-Giemsa solution.

Measurement of serum TGF-β1 levels

The absolute values of both latent and active TGF-β1 in serum were measured by using enzyme immunoassay kits (Genzyme Techné, Minneapolis, Minn), according to the manufacturer's instructions.

CT scans and analysis of airway wall thickness

Helical CT scanning was performed as reported previously.^{20,27} Briefly, a cross-section of the apical bronchus of the right upper lobe was examined at its origin to determine absolute airway thickness, airway wall area (WA), and percentage wall area (WA%; WA/Outer area of the bronchus × 100). WA and absolute airway thickness were normalized for body surface area. The CT data were transferred to a Power PC personal computer and analyzed automatically.²⁷

Pulmonary function

FEV₁ was measured with the use of a Chestac-65V spirometer (Chest MI Corp, Tokyo, Japan). According to American Thoracic Society/European Respiratory Society recommendations,²⁸ spirometry was obtained in triplicate, and the best of 3 reproducible measurements was recorded.

TABLE I. Characteristics of patients with asthma

Male/female sex, n	29/56
Age (y)	55 (15)
Asthma duration (y)	13 (13)
Atopy/nonatopy*, n	51/34
Smoking, never/exsmoker, n	64/21
Asthma severity,† step 1/2/3/4, n	3/30/41/11
FEV ₁ (% predicted)	92 (21)
Total IgE (IU/mL)	153.5 (0.0-11,200)
Dose of inhaled corticosteroid‡ (μg/d)	866 (343)

Data are presented as means with SDs in parentheses, except for total IgE, which is presented as a median with the range in parentheses.

*Atopy was defined by the presence of 1 or more positive specific IgE antibodies against common inhalant allergens.

†Asthma severity was defined according to the Global Initiative for Asthma guidelines.

‡Data from 70 patients who were receiving inhaled corticosteroids, as expressed by the equivalent dose of chlorofluorocarbon–beclomethasone dipropionate.

TABLE II. Frequency of *TGFB1* C-509T polymorphisms

	No.	Percent
Allele frequencies		
C	90	52.9
T	80	47.1
Genotype frequencies		
C/C	22	25.9
C/T	46	54.1
T/T	17	20.0

Statistical analysis

StatView software (SAS Institute, Inc, Cary, NC) was used. Results are expressed as means (SDs), except for total IgE levels, which are presented as medians (ranges). Comparisons of data among genotypes were analyzed by using a χ^2 test, unpaired *t* test, or ANOVA, followed by the Fisher protected least significant difference post-hoc test when significant differences were found. *P* values of less than .05 were considered to indicate statistical significance.

RESULTS**Characteristics of patients**

The characteristics of the patients are presented in Table I. Seventy patients had been treated with inhaled corticosteroids at a dose equivalent to 866 ± 343 μg/d (range, 400-1600 μg/d) of chlorofluorocarbon–beclomethasone dipropionate.

Frequency of *TGFB1* C-509T genotype

Table II summarizes the allele frequencies and genotype distributions. The results were in accordance with those of a healthy Japanese cohort.²⁹

Relations of *TGFB1* C-509T genotype to clinical indices

Age, sex, asthma duration, and the use and dosage of inhaled corticosteroids were similar among the 3 genotypes of C-509T polymorphism (Table III).

Serum TGF-β1 levels were significantly associated with the C-509T polymorphism and were less in patients with CC genotype

TABLE III. Relationship of clinical indices with *TGFB1* C-509T polymorphisms

	CC (n = 22)	CT (n = 46)	TT (n = 17)	P values					
				Three groups*	CC vs CT	CC vs TT	CT vs TT	CC vs CT + TT	CC + CT vs TT
Sex† (M/F)	6/16	17/29	6/11	.72	—	—	—	.43	.91
Age (y)	58 (12)	54 (17)	52 (15)	.37	—	—	—	.18	.41
Asthma duration (y)	12 (12)	14 (13)	13 (17)	.89	—	—	—	.64	.96
Atopy/nonatopy†	15/7	29/17	7/10	.31	—	—	—	.36	.08
Asthma severity†‡ (step 1/2/3/4), n	1/7/13/1	1/16/21/8	1/7/7/2	.57	—	—	—	.61	.47
Dose of inhaled corticosteroid§ (μg/d)	858 (297), n = 19	878 (342), n = 36	847 (416), n = 15	.62	—	—	—	.74	.73
Serum IgE level (IU/mL)	191 (10-1830)	139 (0-11200)	133 (11-2740)	.96	—	—	—	.78	.95
Serum TGF-β1 level (ng/mL)	30 (13)	39 (12)	35 (14)	.03	.01	.31	.21	.02	.64
FEV ₁ (% predicted)	101 (18)	87 (21)	91 (19)	.03	.01	.11	.55	.01	.84
Sputum eosinophils (%)	30 (36)	14 (21)	9 (16)	.04	.03	.03	.60	.01	.22
WA/BSA	20.3 (4.6)	19.4 (6.1)	20.5 (5.3)	.72	—	—	—	.65	.60
WA%	64.8 (5.8)	66.3 (6.9)	66.4 (7.8)	.65	—	—	—	.35	.78
T/√BSA	1.28 (0.18)	1.27 (0.19)	1.31 (0.19)	.72	—	—	—	.98	.44

Data are presented as means with SDs in parentheses, except for total IgE, which is presented as a median with the range in parentheses.

M, Male; F, female; BSA, body surface area; WA%, WA/outer area of the bronchus × 100; T, absolute airway thickness.

*By means of ANOVA or a χ^2 test.

†Differences of distribution among 3 or 2 groups were evaluated by using a χ^2 test.

‡Asthma severity is according to Global Initiative for Asthma guidelines.

§Data of 70 patients taking inhaled corticosteroids are presented, as expressed as the equivalent of chlorofluorocarbon–beclomethasone dipropionate, and comparison of data among genotypes was analyzed by using the Kruskal-Wallis test.

||Data are presented as medians with ranges in parentheses.

than in those with the CT genotype or the CT/TT genotypes combined (Table III and Fig 1). FEV₁ was significantly associated with the polymorphism and was greater in patients with the CC genotype than in those with the CT or CT/TT genotypes combined (Table III and Fig 2). Patients with the CC genotype and those with the TT genotype did not significantly differ in serum TGF-β1 levels or FEV₁, which might be due, at least in part, to the small subject number, especially in the latter genotype. Sputum eosinophil percentage was significantly associated with the polymorphism and was significantly higher in patients with the CC genotype than in those with the CT or TT genotypes or those genotypes combined (Table III and Fig 3). The severity of asthma, atopy, serum IgE levels, and the 3 indices of airway wall thickness were not associated with the C-509T polymorphism (Table III and Fig 4).

DISCUSSION

In our Japanese patients with stable asthma, the T allele of the C-509T polymorphism of the *TGFB1* gene was associated positively with serum TGF-β1 levels and airflow obstruction and negatively with eosinophilic airway inflammation. The polymorphism was not associated with airway wall thickening. To our knowledge, this is the first study of asthmatic patients to examine the relations of a genetic polymorphism associated with asthma to the degree of airway inflammation and airway wall thickening, as assessed by means of CT, an indirect index of airway remodeling.

A positive association between the C-509T polymorphism and asthma risk has been reported in studies of white and Indian populations in the United Kingdom and United States,^{12,14,16} whereas such an association was not noted in Czech or German white subjects or in Hong Kong Chinese.^{13,30,31} An association of this polymorphism with asthma severity has been suggested by one group. Pulleyn et al¹² have found a significant difference in the genotype frequency between patients with mild asthma

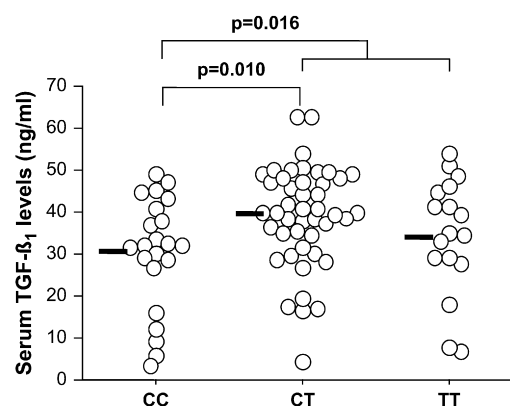


FIG 1. Serum TGF-β1 levels were significantly associated with the C-509T polymorphism ($P = .03$, ANOVA) and were lower in patients with the CC genotype than in those with the CT genotype or the CT/TT genotypes combined. Bars represent means.

and those with severe asthma, which was classified on the basis of symptoms, lung function, and treatment. In the patients with severe asthma, homozygosity for the -509T allele was more common than in those with milder asthma. Mak et al³¹ have shown a similar trend. In contrast, Silverman et al¹⁴ found no association of the C-509T genotype with FEV₁, total IgE level, or blood eosinophil count in the analysis of asthmatic subjects. We have confirmed the results of Pulleyn et al¹² by showing a higher FEV₁ in patients with the CC genotype than in those with the CT genotype or the CT/TT genotypes combined. Although it might be difficult to link a single gene to the severity of asthma, which is a complex disease,³² *TGFB1*, in addition to *IL4* and *IL4R*,³³⁻³⁵ might be a candidate gene. We also found an association between the T allele and higher serum TGF-β1 levels, which is again consistent with

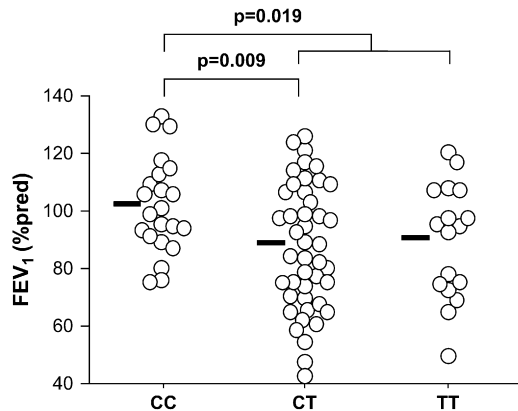


FIG 2. FEV₁ was significantly associated with the C-509T polymorphism ($P = .03$, ANOVA) and was higher in patients with the CC genotype than in those with the CT genotype or the CT/TT genotypes combined. Bars represent means.

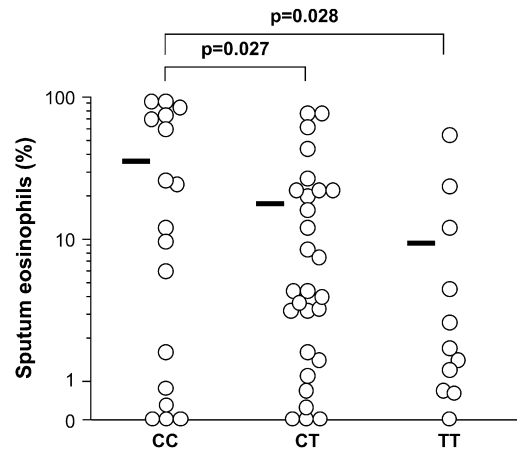


FIG 3. Eosinophil percentage in induced sputum was significantly associated with the C-509T polymorphism ($P = .04$, ANOVA) and was higher in patients with the CC genotype than in those with the CT or TT genotypes or these genotypes combined. Bars represent means.

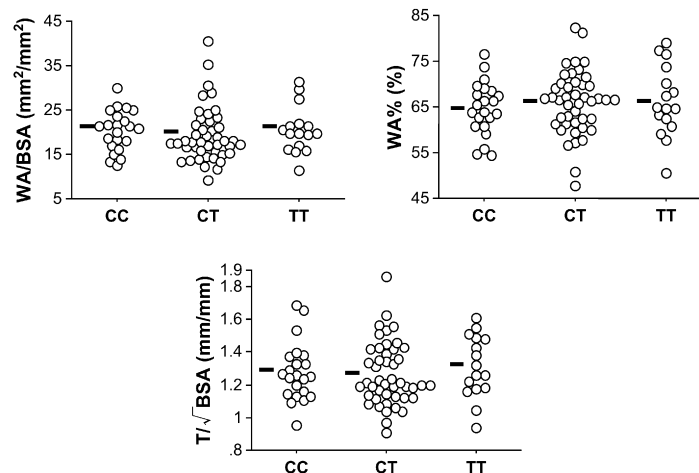


FIG 4. CT indices of airway wall thickness were not associated with the C-509T polymorphism ($P > .6$, ANOVA for all). Bars represent means.

previous reports.^{15,16} An association between the T allele of the C-509T polymorphism and increased levels of serum IgE was not found in our Japanese patients, unlike the study by Hobbs et al,¹¹ which involved both asthmatic and nonasthmatic subjects.

Experimental studies indicate that TGF- β 1 acts as a proinflammatory, as well as an anti-inflammatory, cytokine in the pathophysiology of asthma. Low concentrations of TGF- β 1 induce chemotaxis of eosinophils, whereas higher concentrations reduce eosinophil survival *in vitro*.³⁶ Other studies also indicate that TGF- β 1 inhibits the survival and differentiation of eosinophils.^{37,38} Some animal studies have shown that TGF- β 1 suppresses airway inflammation and airway responsiveness,^{9,10,39} whereas specific inhibition of TGF- β reverses or prevents inflammation and remodeling changes, including smooth muscle proliferation, in other models.^{40,41} In our patients with stable asthma, the sputum eosinophil count was higher in patients with the CC genotype, which was associated with lower serum levels of TGF- β 1, than in patients with the CT or TT genotypes. Increased numbers of eosinophils might support an inhibitory effect of

TGF- β 1 on airway inflammation.^{9,10,36-39} The relation between eosinophils and gene polymorphism or protein levels of TGF- β 1 might not be straightforward, however, because eosinophils are not only a passive follower of TGF- β 1 but also an important source of this cytokine in asthmatic airways.⁶⁻⁸

A polymorphism in the *ADAM33* gene has recently been associated with an accelerated decrease in FEV₁,⁴² but genes involved in airway remodeling, as assessed morphologically, have not been identified in asthma. We have addressed this issue using our validated method for CT.^{18,20,22,27} Airway expression of TGF- β 1 is associated with subepithelial fibrosis in asthmatic patients.^{7,8} The degree of subepithelial fibrosis correlates with airway wall thickness, as assessed by means of CT.^{21,43} We thus assumed that the T allele of the C-509T polymorphism, which is associated with increased TGF- β 1 levels and airflow obstruction in the present and previous studies,^{12,15} might also be related to airway wall thickening but failed to find an association between the polymorphism and CT indices. There might be several explanations for the lack of association. Because airway wall thickening reflects

both structural remodeling and inflammation, such as edema, weaker inflammatory activity associated with the T allele might have attenuated airway wall thickness. Treatment with inhaled corticosteroids might also have affected the results,²² although the use of these drugs was similar among the 3 genotypes. The contribution of small airways to a reduced FEV₁ is another possibility. Such airways cannot be evaluated with the helical CT method used in the present study, but air trapping, as assessed by means of high-resolution CT scanning of the lung, a measure of small airways disease, has been associated with airflow obstruction, airway hypersensitivity, and more severe disease.⁴⁴ Damaged alveolar attachments and decreased elastic fiber content in the adventitia of small airways and peribronchial alveolar septa have been demonstrated in patients with fatal asthma. They can promote airway-parenchymal uncoupling by decreasing the tethering mechanical forces exerted by the surrounding parenchyma, as well as leading to excessive narrowing of smooth muscle.⁴⁵ Phenotypic changes of smooth muscle cells can also be an important mechanism for the small airways involvement in severe asthma.⁴⁶ Because TGF- β is expressed in the asthmatic small airways, as well as in large airways,⁴⁷ TGF- β might be associated with airflow obstruction by exaggerating these remodeling changes of small airways. This assumption, however, needs to be clarified further in future studies.

In conclusion, the T allele of the *TGFB1* C-509T polymorphism is related to augmented airflow obstruction but attenuated eosinophilic inflammation, in addition to the known increase in serum TGF- β 1 levels. The relation with increased airway obstruction is apparently not attributed to the thickening of central airway walls. The relation with reduced eosinophilic inflammation might reflect the inhibitory effect of TGF- β 1 on airway inflammation. The C-509T polymorphism is involved in the pathophysiology of asthma in a complex way, presumably because TGF- β 1 has diverse functions and interacts with various cells or humoral factors in persons with asthma.^{2,4,5} Although TGF- β 1 has been regarded as one of the future therapeutic targets in asthma,^{2,40,41} treatment strategy against TGF- β 1 might require consideration of its functional complexity.

We thank Goro Saji and Ryuzo Tanaka for their radiologic technical support.

Clinical implications: A treatment strategy targeting TGF- β 1 might require consideration of its functional complexity.

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