

Analysis of TGF- β_1 gene polymorphisms in Hong Kong Chinese patients with asthma

Judith C. W. Mak, PhD,^a Helen C. M. Leung, MPhil,^a Siu P. Ho, BSc,^a Barbara K. W. Law, BSc,^a Alice S. S. Ho, MBChB,^b Wah K. Lam, MD,^a Mary S. M. Ip, MD,^a and Moira M. W. Chan-Yeung, MB^a Hong Kong SAR, China

Background: The C-509T polymorphism of TGF- β_1 gene has been associated with asthma and atopy in white populations. **Objective:** We investigated the association between asthma and previously identified polymorphisms at C-509T and T869C of the TGF- β_1 gene among 250 Chinese patients with asthma and 308 healthy controls in Hong Kong.

Methods: Genotyping was performed on peripheral blood genomic DNA by using PCR-RFLP.

Results: There were no differences in the frequencies of genotypes and alleles between patients and controls. The C-509T and T869C polymorphisms were in tight linkage disequilibrium ($P < .01$). Among atopic subjects, significant differences were found in genotype and allele frequencies for T869C polymorphism between patients and controls ($P = .014$ and $P = .019$, respectively), and individuals bearing the CC genotype were associated with increased risk for the development of asthma (odds ratio, 2.58; 95% CI, 1.17-5.66; $P = .018$) after adjusting for age, sex, and smoking status. Individuals with asthma bearing the CT genotype of the C-509T polymorphism had significantly increased risk for severe airflow obstruction compared with individuals who had mild obstruction (odds ratio, 4.00; 95% CI, 1.06-15.08; $P = .035$).

Conclusion: Our results indicate that the polymorphisms at C-509T and T869C of the TGF- β_1 gene are associated with asthma susceptibility in atopic subjects of the Hong Kong Chinese population, and the C-509T polymorphism may play a role in the pathogenesis of airflow obstruction. (J Allergy Clin Immunol 2006;117:92-6.)

Key words: Transforming growth factor- β_1 , polymorphism, asthma, disease severity, Hong Kong Chinese population

Asthma is a chronic inflammatory airway disease that affects children and adults of all ages.¹ The association between polymorphisms of relevant genes and features of asthma has been an important advance over the past decade.² TGF- β_1 is a multifunctional cytokine that has been implicated in the pathogenesis of asthma. Increased TGF- β_1 expression has been found in bronchoalveolar

Abbreviation used

OR: Odds ratio

lavage fluid, bronchial biopsies, and plasma from patients with asthma.³⁻⁵ TGF- β_1 mRNA levels in eosinophils are also significantly increased in patients with asthma.^{6,7} It is postulated that TGF- β_1 may play an important role in airway remodeling.⁸ TGF- β_1 is expressed by many different cell types including airway epithelial cells, eosinophils, T_H2 lymphocytes, macrophages, and fibroblasts. One of the features of airway remodeling is subepithelial fibrosis, which is increased in patients with severe asthma.⁷ TGF- β_1 has been implicated in several aspects of fibrosis, including the deposition of extracellular matrix proteins such as collagens and fibronectin.⁹⁻¹¹ Studies in rodent models of asthma indicate that TGF- β_1 may have both anti-inflammatory and profibrotic effects.¹¹⁻¹³

The TGF- β_1 gene is located on chromosome 19q13 and has been cloned and sequenced.^{14,15} There are several registered polymorphisms within TGF- β_1 and its promoter¹⁶ that might be functional and might be associated with airway diseases.^{17,18} Two polymorphisms of the TGF- β_1 gene, C-509T and T869C, are in strong linkage disequilibrium. The results of association studies on these polymorphisms and asthma risk, however, have been inconsistent. A promoter polymorphism at C-509T has been shown to be associated with elevated plasma TGF- β_1 levels,¹⁹ total IgE,²⁰ and asthma risk.²¹⁻²³ On the other hand, 2 studies suggested no association between the polymorphisms at C-509T and T869C and asthma risk.^{24,25} In this study, we sought to detect polymorphisms inside the gene (T869C) and its promoter (C-509T) of TGF- β_1 to compare the genotype and allele frequencies between the patients and control groups. Because haplotype analysis, testing associations using at least 2 common polymorphisms, will demonstrate genetic influences that are not detected by the analysis of single polymorphisms, we also checked the linkage disequilibrium and compared the haplotypes between the common polymorphisms in Hong Kong Chinese population.

METHODS

Study subjects

Two hundred fifty consecutive Chinese patients with doctor-diagnosed asthma defined by the American Thoracic Society were

From ^athe Division of Respiratory Medicine, Department of Medicine, University of Hong Kong; and ^bAlice Ho Miu Ling Nethersole Hospital. Supported by a grant from the Hong Kong Research Grant Council (HKU7223/01M).

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Reprint requests: Judith C. W. Mak, PhD, Department of Medicine, Room 804, Administration Block, Queen Mary Hospital, Hong Kong SAR, China. E-mail: judithmak@hkucc.hku.hk.

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TABLE I. Characteristics of asthma patients and healthy controls

	Controls	Patients
N	308	250
Male, n (%)	211 (68.5)	106 (42.4)*
Age†	51.5 ± 15.2	41.0 ± 16.1*
Smoking, n (%)		
Nonsmoker	163 (52.9)	192 (77.1)*
Ever-smoker	145 (47.1)	57 (22.9)*
Atopy, n (%)		
Atopic	100 (32.5)	157 (67.7)*
Nonatopic	208 (67.5)	75 (32.3)*
Duration of asthma†	NA	16.6 ± 14.8
FEV ₁ % predicted†	108.2 ± 15.6	81.3 ± 23.5*
Forced vital capacity % predicted†	104.6 ± 14.7	96.6 ± 21.0*

* $P < .001$ for differences between patients with asthma and healthy controls by t test or χ^2 test.

†Mean ± SD.

recruited from the asthma clinic at Queen Mary Hospital in Hong Kong (participation rate, 95%). All patients were stable at the time of recruitment without acute exacerbation for at least 3 months receiving treatment in the clinic with inhaled corticosteroids and/or bronchodilator when necessary, and had evidence of reversible airflow limitation with a significant (>12% change in FEV₁ or forced vital capacity) response to acute inhaled bronchodilator treatment.²⁶ The patients were categorized into severity groups by using level of FEV₁ % predicted as having mild (FEV₁ > 80%), moderate (FEV₁ 60% to 80%), or severe (FEV₁ < 60%) airflow obstruction.

A total of 308 healthy subjects without respiratory symptoms with normal lung function served as controls. They represented 45% of subjects who participated in a study for reference value of lung function in Hong Kong Chinese recruited from the general population by using random digit dialing.

All individuals had lung function tests and allergy skin prick test using 6 common allergens (house dust mite, cat and dog dander, cockroach, mixed grass pollen, and mixed molds) together with histamine 1 mg/mL and normal saline as a positive and a negative control, respectively. Atopy was defined as the presence of positive skin test reaction (≥ 3 mm greater than reaction to saline) to 1 or more of the common allergens.²⁷ In addition, a blood sample was taken from each participant for genotyping in this study. Ever-smokers were defined as subjects who had ever smoked at least 1 cigarette a day for 1 year or more, whereas nonsmokers were lifelong nonsmokers who had never smoked in their life. Patients and controls were ethnically Chinese originated from Hong Kong or Guangdong province of Southern China. The Ethics Committee of the University of Hong Kong approved the study protocol, and all of the participants provided written informed consent.

Genotyping

DNA was extracted from buffy coats by using a commercially available DNA extraction kit (Qiagen, Hilden, Germany). The genotypes of C-509T and T869C of TGF- β_1 gene were determined by PCR-RFLP as described previously.²¹ The T869C variant was amplified by using the GC-rich PCR system (Roche, Mannheim, Germany). The PCR products were digested with restriction enzyme DdeI for C-509T and BglII for T869C, respectively, and electrophoresed on 4% agarose gels. Samples were genotyped from the digested band sizes.

TABLE II. Genotype distributions, allele frequencies, and estimated haplotype frequencies of C-509T and T869C polymorphisms of TGF- β_1 gene in healthy controls and patients with asthma

	Controls N (%)	Patients N (%)	P value
C-509T genotype			
CC	51 (16.6)	46 (18.5)	
CT	155 (50.3)	109 (44.0)	
TT	102 (33.1)	93 (37.5)	.326
Allele C	257 (41.7)	201 (40.5)	
T	359 (58.3)	295 (59.5)	.687
T869C genotype			
TT	70 (22.7)	49 (19.8)	
TC	151 (49.0)	108 (43.5)	
CC	87 (28.2)	91 (36.7)	.105
Allele T	291 (47.2)	206 (41.5)	
C	325 (52.8)	290 (58.5)	.057
Haplotype			
C-509T T869C	234 (38.1)	198 (39.8)	<.001
C T	22 (3.6)	3 (0.6)	
C C	56 (9.1)	7 (1.4)	
T T	302 (49.2)	290 (58.2)	
T C			

Haplotype estimation and statistical analysis

Haplotype frequencies for sets of alleles on the basis of the maximum likelihood method, pairwise linkage disequilibrium coefficients (D'), and Hardy-Weinberg equilibrium were estimated by using SNPAnalyzer program (Istec Inc, Ilsan-gu, Korea; <http://istech21.com/>). SPSS for Windows version 11.0 statistical package (SPSS, Chicago, Ill) was used for statistical analyses. Demographic data were compared between groups by using χ^2 analysis or ANOVA as appropriate. Differences in genotype and allele frequencies between patients and controls were tested by using χ^2 analyses for a 2×2 table or by using the Fisher exact test. Unconditional logistic regression models were applied to estimate odds ratio (ORs) and 95% CIs for the genetic risk factor in the total study population and in the subgroups of atopic status, adjusted for age, sex, and smoking status. The differences were considered significant at $P < .05$.

RESULTS

Table I summarizes the characteristics of the study population. In total, we genotyped 558 subjects consisting of 250 patients with asthma and 308 healthy controls. Patients with asthma were significantly younger and more likely to be female, nonsmokers (77%), and atopic (68%). The FEV₁ % predicted values were significantly decreased in patients with asthma compared with healthy controls.

Table II summarizes the genotype distributions, allele frequencies, and estimated haplotype frequencies of TGF- β_1 gene polymorphisms at C-509T and T869C in healthy controls and patients with asthma. The genotype distributions of both polymorphisms were in Hardy-Weinberg equilibrium. We found no significant differences in genotype distributions and allele frequencies between patients with asthma and controls without or with stratification by smoking status (data not shown). The C-509T and T869C polymorphisms were found to be in tight linkage disequilibrium ($D' = 0.9830$ and 0.8375 for subjects with asthma and control subjects, respectively), and the major haplotypes for these 2 loci were

TABLE III. Risk associated with C-509T and T869C polymorphisms of TGF- β_1 gene in patients with asthma compared with controls by atopic status

Genotype	Nonatopic			Atopic		
	Controls N (%)	Patients N (%)	Adjusted* OR (95% CI)	Controls N (%)	Patients N (%)	Adjusted* OR (95% CI)
C-509T genotype CC	34 (16.3)	11 (15.1)	1	17 (17.0)	30 (19.1)	1
CT	100 (48.1)	36 (49.3)	1.01 (0.44-2.30)	55 (55.0)	62 (39.5)	0.69 (0.32-1.48)
TT	74 (35.6)	26 (35.6)	1.21 (0.51-2.88)	28 (28.0)	65 (41.4)	1.44 (0.64-3.25)
Allele C	168 (40.4)	58 (39.7)	1	89 (44.5)	122 (38.9)	1
T	248 (59.6)	88 (60.3)	1.12 (0.74-1.68)	111 (55.5)	192 (61.1)	1.33 (0.90-1.96)
T869C genotype TT	46 (22.1)	13 (17.3)	1	24 (24.0)	31 (19.9)	1
TC	99 (47.6)	37 (49.3)	1.31 (0.61-2.80)	52 (52.0)	60 (38.5)	1.00 (0.49-2.06)
CC	63 (30.3)	25 (33.3)	1.44 (0.64-3.25)	24 (24.0)	65 (41.7)	2.58† (1.17-5.66)
Allele T	191 (45.9)	63 (42.0)	1	100 (50.0)	122 (38.4)	1
C	225 (54.1)	87 (58.0)	1.19 (0.80-1.78)	100 (50.0)	196 (61.6)	1.73‡ (1.17-2.56)

*Adjusted for age, sex, and smoking status.

† $P = .018$; ‡ $P = .006$.

C-T and T-C. There were significant differences ($P < .001$) in haplotype frequencies among patients with asthma and controls. The C-C and T-T haplotypes were significantly associated with asthma, and those bearing the C-C and T-T haplotypes had a protective role (OR, 0.15; 95% CI, 0.06-0.33; $P < .001$; and OR, 0.16; 95% CI, 0.05-0.55; $P < .001$, respectively).

Among atopic subjects, significant differences were observed in genotype and allele distribution for T869C polymorphism between patients with asthma and controls ($P = .014$ and $P = .019$, respectively), and those bearing the CC genotype had a more than 2-fold significantly increased risk for the development of asthma (OR, 2.58; 95% CI, 1.17-5.66; $P = .018$; Table III) after adjusting for age, sex, and smoking status. In the C-509T polymorphism, we also found significant differences in genotype frequencies between atopic patients with asthma and atopic controls ($P = .041$; Table III), but not allele frequencies.

Differences were found in the genotype frequencies but not in allele frequencies among patients with asthma with different degree of airflow obstruction in comparison with controls at C-509T ($P = .053$) and T869C ($P = .023$; Table IV). In the C-509T polymorphism, patients with asthma bearing the CT genotype were found to have a 4-fold significantly increased risk for severe airflow obstruction compared with subjects who had mild obstruction (OR, 4.00; 95% CI, 1.06-15.08; $P = .035$). On the other hand, the increased risk of patients with asthma bearing the TC genotype of T869C polymorphism for severe airflow obstruction compared with subjects who had mild obstruction was at borderline significant difference (OR, 3.85; 95% CI, 1.02-14.60; $P = .055$).

Logistic regression analysis on combined C-509T and T869C genotypes for all subjects revealed that CT/TT or TT/TC was associated with a decreased risk for the development of asthma (OR, 0.18; 95% CI, 0.04-0.73; $P = .017$; and OR, 0.07; 95% CI, 0.01-0.54; $P = .011$ for CT/TT and TT/TC, respectively; Table V) after adjusting for age, sex, smoking, and atopic status. However, we were unable to calculate the frequencies of combined C-

509T and T869C genotypes after stratification on the basis of smoking and atopic status because of the small numbers.

DISCUSSION

In this study, we found differences in the frequencies of the T and C alleles and TT and CC genotype of the TGF- β_1 C-509T polymorphism of the Hong Kong Chinese population and those reported for white populations. The frequencies of the T allele and TT genotype of the TGF- β_1 C-509T polymorphism in white and Indian populations were low,²¹⁻²³ whereas those of the Chinese population in Hong Kong were higher, similar to those reported in Taiwanese and Japanese populations.^{28,29} The frequencies of the C allele and CC genotype of the TGF- β_1 T869C polymorphism in 2 white populations were lower,¹⁸ and those in Hong Kong Chinese were similar to those reported in Taiwanese and Japanese populations.^{28,29} Differences in the prevalence of the C-509T and T869C polymorphisms may be attributable to the differences in genetic background between races or to differences in linkage disequilibrium (the association between polymorphisms on a chromosome). In this study, we found that C-509T and T869C loci were in tight linkage disequilibrium, and the major haplotypes were C-T and T-C, consistent with previous findings.^{28,30}

The lack of an association of the C-509T polymorphism of the TGF- β_1 gene with asthma in our population was contrary to those reported in the United Kingdom and the United States white and Indian populations,²¹⁻²³ but in agreement with the Czech and German white populations.^{24,25} We found, however, that among atopic subjects, the homozygous CC genotype at T869C was associated with increased risk of asthma after adjusting for age, sex, and smoking status, an association that has not been reported previously.

In this study, we found that the CT genotype at the C-509T polymorphism was associated with severity of airflow obstruction. However, in patients with cystic fibrosis,

TABLE IV. Genotype frequencies of C-509T and T869C polymorphisms of TGF- β_1 gene in patients with asthma classified according to severity on the basis of the level of lung function

Polymorphic sites	Control N (%)	Severity of airflow obstruction			P value
		Mild N (%)	Moderate N (%)	Severe N (%)	
C-509T CC	51 (16.6)	24 (21.8)	10 (18.9)	3 (9.4)	.053
CT	155 (50.3)	36 (32.7)	26 (49.1)	18 (56.2)	
TT	102 (33.1)	50 (45.5)	17 (32.0)	11 (34.4)	
T869C TT	70 (22.7)	26 (23.4)	10 (18.9)	3 (10.0)	.023
TC	151 (49.1)	36 (32.4)	27 (50.9)	16 (53.3)	
CC	87 (28.2)	49 (44.2)	16 (30.2)	11 (36.7)	

TABLE V. Distribution frequencies of C-509T and T869C genotypes in combination and risk of asthma with reference to the wild-type genotypes

Combination of C-509T and T869C genotypes	Controls N (%)	Patients with asthma N (%)	Adjusted* OR (95% CI)
CC/TT	46 (14.9)	45 (18.3)	1.0
CC/TC	5 (1.6)	1 (0.4)	NA
CT/TT	21 (6.8)	3 (1.2)	0.18† (0.04-0.73)
CT/TC	119 (38.6)	104 (42.3)	0.91 (0.53-1.54)
CT/CC	15 (4.9)	0	NA
TT/TC	27 (8.8)	2 (0.8)	0.07† (0.01-0.54)
TT/CC	72 (23.4)	91 (37.0)	1.43 (0.82-2.50)
TT/TT	3 (1.0)	0	NA

*Adjusted for age, sex, smoking, and atopic status.

† $P \leq .017$.

the TT genotype of T869C polymorphism was associated with a more rapid deterioration in lung function.³¹ TGF- β_1 has been implicated in several aspects of fibrosis, including the deposition of extracellular matrix proteins such as collagens and fibronectin.⁹⁻¹¹ In asthma, subepithelial airway fibrosis correlates with asthma severity.⁷ Polymorphisms of cytokine genes such as IL-4 and TNF- α and β_2 -adrenergic receptor gene have been identified to be associated with severity of asthma (on the basis of FEV₁).³²⁻³⁶

Both polymorphisms investigated in this study, C-509T and T869C polymorphisms in the TGF- β_1 gene, have been reported to be associated with the transcriptional activity of the gene or the serum level of the gene product. The C-509T polymorphism was associated with significantly lower circulating concentration of TGF- β_1 in those with the CC genotype compared with other genotypes in white women and Indian populations.^{19,23} On the other hand, the CC genotype of the T869C polymorphism was associated with higher TGF- β_1 concentration than other genotypes in Japanese,^{37,38} but not in white subjects.³⁹ In this study, we did not measure plasma TGF- β_1 levels to investigate their relationship with C-509T or T869C polymorphisms. A scenario is that TGF- β_1 is harmful in the airways of individuals with asthma; its sustained elevation may stimulate airway remodeling. Under this paradigm, genetic variants that are associated with elevated TGF- β_1 levels, such as the T allele of C-509T and the C allele of T869C, would be expected to be associated

with higher asthma prevalence or increased asthma severity. Our findings of higher frequencies of the TT genotype of C-509T and the CC genotype of T869C alone and in combination may support a harmful role of TGF- β_1 in asthma. However, the possible TGF- β_1 pathogenetic mechanism needs further clarification.

For the majority of multifactorial diseases, such as asthma, it is unlikely that a single polymorphism in a single gene could alter expression or function of specific proteins to produce pathologic phenotypes. It seems more likely that the sum of effects of different single nucleotide polymorphisms in a gene produces some changes in expression/function. Despite their tight linkage disequilibrium between the C-509T and T869C loci, separate associations of the 2 single nucleotide polymorphisms with specific phenotypes were found, suggesting that the Hong Kong Chinese population is heterogeneous both in terms of asthma risk and C-509T or T869C genotypes, perhaps as a result of an older population admixture in southern China, although this remains entirely speculative. It cannot be excluded that other polymorphisms or complex haplotypes within the promoter and coding region of the TGF- β_1 gene or in adjacent genes might contribute to the genetic inheritance of asthma. One limitation of this study is that we used lung function measurement as an indicator of disease severity; because it is a cross-sectional study, lung function measurement alone may not be a good indicator of severity of asthma. However, the patients were recruited when they were stable and

had no acute exacerbation for at least 3 months, and their best FEV₁ (% predicted) was used in the analysis.

In conclusion, the C-509T and T869C polymorphisms of the TGF- β ₁ gene are associated with asthma susceptibility in atopic subjects of the Hong Kong Chinese population, and the C-509T polymorphism is associated with more severe disease, suggesting that it might play a role in the pathogenesis of airflow obstruction. Our findings should encourage further studies of the functional relationship of plasma TGF- β ₁ levels and C-509T or/and T869C polymorphisms.

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