

TGF β 1 haplotypes and asthma in Indian populations

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Background: Asthma is a complex disorder of the airways of the lungs. TGF- β 1 plays a key role in airway remodeling and asthma by having both proinflammatory and anti-inflammatory activities, making *TGF β 1* an important candidate gene to study.

Objective: To investigate the association of *TGF β 1* gene polymorphisms with asthma.

Methods: A case-control study was designed for identifying polymorphisms and haplotypes associated with asthma and associated phenotypes. We have verified our results in 2 independent cohorts collected from northern (number of patients, 187; number of controls, 187) and western India (number of patients, 209; number of controls, 190). We measured the serum TGF- β 1 levels of selected individuals and correlated them with genotypes and haplotypes.

Results: A novel (CT)_n(CA)_m repeat polymorphism (BV209662) 24.9 kb upstream of *TGF β 1* was identified. A significant association was seen at the level of alleles and genotypes with asthma in the 2 cohorts studied independently ($P < .05$). Interestingly, a novel 3-locus haplotype, 23_G_T, was found to be significantly associated with asthma ($P = .00001$ in cohorts A and B) as well as with higher serum TGF- β 1 level ($P = .01$). On the other hand, a novel haplotype, 22_G_C, was negatively associated with asthma ($P = .00001$ for cohorts A and B) and with lower serum TGF- β 1 level ($P = .0019$).

Conclusion: This is the first study identifying novel risk and protective haplotypes—23_G_T and 22_G_C, respectively—in the *TGF β 1* gene that are associated with asthma. We also demonstrate the functional significance of these haplotypes with serum TGF- β 1 levels. These results would be valuable in elucidating the role of TGF- β 1 in asthma pathogenesis. (*J Allergy Clin Immunol* 2005;115:527-33.)

Key words: Asthma, case-control study, haplotype, Indian population, serum IgE, *TGF β 1*

TGF- β 1 plays an important role in airway remodeling, an established pathological feature in asthma.^{1,2} It is

Abbreviations used

KS: Kolmogorov-Smirnov
LD: Linkage disequilibrium
OR: Odds ratio
SNP: Single nucleotide polymorphism
SPT: Skin prick test

implicated in several aspects of fibrosis³ wherein sub-epithelial fibrosis is increased in patients with severe asthma.⁴ In addition, it decreases synthesis of enzymes that degrade the extracellular matrix, such as collagenase and stromelysin, and increases synthesis of inhibitors of these enzymes, including tissue inhibitor of metalloproteinase 1 and plasminogen activator inhibitor type 1.⁴ TGF- β 1 mRNA levels in eosinophils are increased in patients with severe asthma compared with mild asthma.^{3,5} In contrast, it also prevents the development of allergic inflammation through the capacity to inhibit IgE synthesis and through inhibition of basophil and eosinophil proliferation.⁶ In addition, it abrogates the survival effects of hematopoietins on eosinophils and thereby induces their apoptosis.⁷ This complex mix of proinflammatory and anti-inflammatory activities makes *TGF β 1* a promising candidate gene for asthma.

The *TGF β 1* gene is located on chromosome 19q13.1-13.2⁸ and has recently been linked to mite sensitivity.⁹ Studies performed in different populations have identified various polymorphisms, such as -988C/A, -800G/A, -509C/T, 869T/C, and 915G/C. Earlier studies also reported a strong linkage disequilibrium (LD) among -509C/T, 869T/C, and 915G/C.^{10,11} Out of these single nucleotide polymorphisms (SNPs), the C to T transition at the -509 position has been found to be associated with elevated IgE levels¹² and TGF- β 1 levels.¹³ In another study, this polymorphism was associated with asthma severity.¹¹ In the same study, 4 other polymorphisms, -988C/A, -800G/A, 869T/C, and 915G/C, were assessed for association with asthma, but no significant association was found. A similar study in the Czech population showed no association with asthma.¹⁴

Thus, both genetic and biochemical evidence indicates that *TGF β 1* is a potential candidate gene for disease pathogenesis and/or susceptibility to asthma. To elucidate its role in asthma genetics, we have performed a case-control study in 2 independent cohorts of patients with asthma and controls. Here, we have genotyped a novel repeat

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TABLE I. Demographic profile of the patient and control groups

	Controls		Patients	
	Cohort A	Cohort B	Cohort A	Cohort B
Native place*	North India	West India	North India	West India
Mean age, y	24.01 (± 0.95)	29.35 (± 1.00)	28.42 (± 0.97)	34.22 (± 1.01)
Sex ratio (M:F)	0.58:0.42	0.56:0.44	0.52:0.48	0.53:0.47
Familial history of asthma/atopy	None	None	All	All
Smoking history	None	None	None	None
% Reversibility from baseline FEV ₁ (after β_2 -agonist use)	ND	ND	>15%	>15%
Log mean total serum IgE (IU/mL)	2.41 $\pm$ 0.07	2.40 $\pm$ 0.05	2.85 $\pm$ 0.07	2.87 $\pm$ 0.04
Self-reported history of allergies	None	None	All	All

ND, Not done.

*Patients in cohort A were recruited from the states of Delhi, Haryana, and Punjab, whereas patients in cohort B were recruited from Gujarat, Maharashtra, and Rajasthan. Control samples were taken from all of these states. Parentheses contain the values for SE.

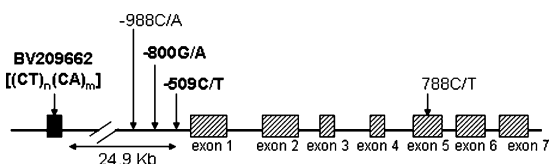


FIG 1. Gene structure and polymorphisms investigated in the *TGFβ1* gene. The positions marked in **bold** were found polymorphic in the Indian population.

(accession number BV209662) and 2 SNPs, $-800G/A$ and $-509C/T$, encompassing a region of 24.7 kb, and have analyzed the association of these polymorphisms independently and at the level of haplotype with asthma and also with serum TGF- $\beta 1$ levels.

METHODS

Patients and healthy volunteers

Unrelated patients were recruited from various collaborating hospitals in northern India in cohort A ($N = 187$) and western India in cohort B ($N = 209$; Table I). Ethical approval was obtained from the review board of each hospital. Written informed consent was obtained from all individuals participating in the study. Asthma in the recruited study population was defined by clinical history and was validated later by interview questions (details of environmental factors, family history of asthma/atopy, the geographical region of origin, and migration status). Patients (mean age, 28.42 years $\pm$ 0.97 years and 34.22 years $\pm$ 1.01 years for cohorts A and B, respectively) were diagnosed with asthma on the basis of National Asthma Education and Prevention Program (Expert Panel Report 2) guidelines¹⁵ and were examined for a self-reported history of breathlessness and wheezing. The clinical parameters are summarized in Table I. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% (by using albuterol/salbutamol). Fifteen common environmental allergens were used for the skin prick test (SPT). Atopy was defined as having wheal reaction equal to or greater than histamine (3-mm diameter). All subjects with asthma had a positive reaction to at least 1 antigen used for the SPT. Total serum IgE levels were estimated for all individuals by using ELISA,¹⁶ except a few individuals (<10%) for whom sera were not available.

Healthy volunteers (normal controls; $N = 187$ and 190 in cohorts A and B, respectively) were recruited on the basis of the criteria of

having no symptoms or history of allergic diseases. Individuals having a history of smoking and parasitic/helminthic infestations in the past were excluded from the study. All control individuals recruited for the study were screened negative for all of the allergens used to perform SPT.

In this study, because the samples were collected from individuals on the basis of their family history, origin, and migration status, the error caused by stratification is presumably minimized (Table I). Indeed, we have established the genetic homogeneity between the 2 groups by genotyping multiple loci, as yet unlinked to asthma or related atopic disorders.¹⁷ Also, all individuals were age-matched and sex-matched (Table I).

Serum TGF- $\beta 1$ level measurement

We used the TGF- $\beta 1$ ELISA system (R&D Systems, Minneapolis, Minn) as per the manufacturer's instructions to determine total TGF- $\beta 1$ levels (active and latent form) from serum.

Genomic DNA preparation

DNA was isolated from peripheral white blood cells by using the modified salting out method as described¹⁶ and was stored at -20°C .

SNP genotyping

The $-509C/T$ and $-800G/A$ polymorphisms (Fig 1) were investigated by using primers detailed in Table II. The $-509C/T$ polymorphism was assessed by using *Bsu* 36 I restriction endonuclease digestion. The other 3 polymorphisms were studied by using SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, Calif) as per the manufacturer's instructions. These samples were subsequently electrophoresed by using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The results were analyzed by using the ABI Prism GeneScan and Genotyper (Applied Biosystems).

Genotyping of repetitive sequences in and around *TGFβ1*

A $(CT)_n(CA)_m$ repeat (BV209662), 24.9 kb upstream of the gene, was identified by using the RepeatMasker software (<http://www.repeatmasker.org>) and validated for distribution in our population. PCR was performed in a total volume of 15 μL containing 25 ng genomic DNA, 4 pmol each of a 6-carboxyfluorescein-labeled forward primer and a nonlabeled reverse primer (Table II), 1.5 mmol/L MgCl_2 , 0.25 mmol/L of each deoxynucleotide triphosphate,

TABLE II. Primer details for PCR of various loci

Primer	Number of bases	Sequence	Amplified product size
(CT) _n (CA) _m FP (6-FAM-labeled)	20 bases	5' CCC CAC TAA GGC CAG CAT CT 3'	363 bp
(CT) _n (CA) _m RP	19 bases	5' TTA AGC CCG GGA GGT CAA G 3'	
–800G/A FP	20 bases	5' CCC GGC TCC ATT TCC AGG TG 3'	388 bp
–800G/A RP	20 bases	5' TGC TCT TGA CCA CTG TGC CA 3'	
–509C/T FP	20 bases	5' CAG ACT CTA GAG ACT GTC AG 3'	418 bp
–509C/T RP	19 bases	5' GTC ACC AGA GAA AGA GGA C 3'	
–988C/A FP	20 bases	5' TGC CAG CTT GCA GGC TAT GG 3'	232 bp
–988C/A RP	20 bases	5' GGA GCA GCA GGC CGA TCT CC 3'	
788C/T FP	19 bases	5' TTC CCT CGA GGC CCT CCT A 3'	294 bp
788C/T RP	20 bases	5' GCC GCA GCT TGG ACA GGA TC 3'	

6-FAM, 6-Carboxyfluorescein; FP, forward primer; RP, reverse primer.

TABLE III. Allele frequencies of (CT)_n(CA)_m polymorphism in *TGFβ1* gene*

(CT) _n (CA) _m allele	Number of individuals									
	Cohort A					Cohort B				
	Cases (%)	Controls (%)	OR	99% CI	P value†	Cases (%)	Controls (%)	OR	99% CI	P value‡
19	1 (0.27)	1 (0.27)				—	—			
20	1 (0.27)	8 (2.14)				1 (0.24)	2 (0.53)			
21	183 (48.93)	96 (25.67)	2.77	1.85-4.16	.0000	183 (43.78)	120 (31.58)	1.69	1.15-2.47	.0003
22	44 (11.76)	151 (40.37)	0.20	0.12-0.32	.0000	59 (14.11)	129 (33.95)	0.32	0.20-0.50	.0000
23	116 (31.02)	62 (16.58)	2.26	1.43-3.58	.0000	124 (29.67)	49 (12.89)	2.85	1.76-4.6	.0000
24	27 (7.22)	51 (13.64)	0.49	0.26-0.94	.0038	45 (10.77)	70 (18.42)	0.53	0.31-0.90	.0020
25	2 (0.53)	5 (1.34)				6 (1.44)	10 (2.63)			

*Numbers in parentheses indicate the frequency (%).

For Bonferroni correction, †α = 0.007 and ‡α = 0.008.

TABLE IV. Distribution of allele and genotype frequencies for –800G/A and –509C/T polymorphisms in 2 cohorts studied*

Alleles	Number of individuals									
	Cohort A					Cohort B				
	Patients	Controls	OR	99% CI	P value	Patients	Controls	OR	99% CI	P value
–509										
C	232 (62.03)	265 (70.86)	0.67	0.45-1.00	.01	243 (58.13)	287 (75.53)	0.45	0.30-0.67	.0000
T	142 (37.97)	109 (29.14)				175 (41.87)	93 (24.47)			
–800										
A	39 (10.43)	25 (6.68)			NS	24 (5.74)	20 (5.26)			NS
G	335 (89.57)	349 (93.32)				394 (94.26)	360 (94.74)			
Genotypes										
–509										
CC	64 (34.22)	94 (50.27)	0.51	0.30-0.89	.0016	73 (34.93)	112 (58.95)	0.37	0.22-0.64	.0000
CT	104 (55.61)	77 (41.18)	1.79	1.04-3.07	.0051	97 (46.41)	63 (33.16)	1.74	1.02-2.98	.0068
TT	19 (10.16)	16 (8.56)				39 (18.66)	15 (7.89)			
–800										
AA	0 (0.00)	1 (0.53)			NS	0 (0.00)	0 (0.00)			NS
GA	39 (20.86)	23 (12.30)				24 (11.48)	20 (10.53)			
GG	148 (79.14)	163 (87.17)				185 (88.52)	170 (89.47)			

NS, Nonsignificant.

*Numbers in parentheses indicate the frequency (%).

0.03 U/μL Taq DNA polymerase (Bangalore Genie, Bangalore, India), and the buffer recommended by the supplier. Fragment lengths were determined by using the GeneScan Analysis Software version 3.7 (Applied Biosystems). The number of repeats was determined by sequencing the PCR fragments from individuals (N = 5) homozygous for 1 allele.

Statistical analysis

The Kolmogorov-Smirnov (KS) test was used to test the association of the allele size distribution of (CT)_n(CA)_m repeat with disease. The χ^2 test was used to test the genotypic and allelic association of the different loci studied with asthma. Odds ratios (ORs) were calculated for the alleles and genotypes. ANOVA was performed to

TABLE V. Multiple logistic regression analysis of the *TGFβ1* polymorphisms for risk of asthma*

Variable	Cohort A			Cohort B		
	LR χ^2	df	P value	LR χ^2	df	P value
Model 1						
(CT) _n (CA) _m genotype	64.89	17	.00001	41.31	15	.0003
–800 genotype	7.49	2	.02	0.076	1	.78
–509 genotype	8.59	2	.013	5.56	2	.062
Age	10.57	1	.001	9.15	1	.002
Sex	0.77	1	.38	6.13	1	.013
Model 2						
(CT) _n (CA) _m genotype	79.95	17	.00001	50.76	17	.00001
–800 genotype	6.02	2	.049	0.11	1	.73
–509 genotype	7.13	2	.028	10.88	2	.004

*Model 1: There was controlling for age and sex. Model 2: There was no controlling for age and sex.

LR, Likelihood ratio.

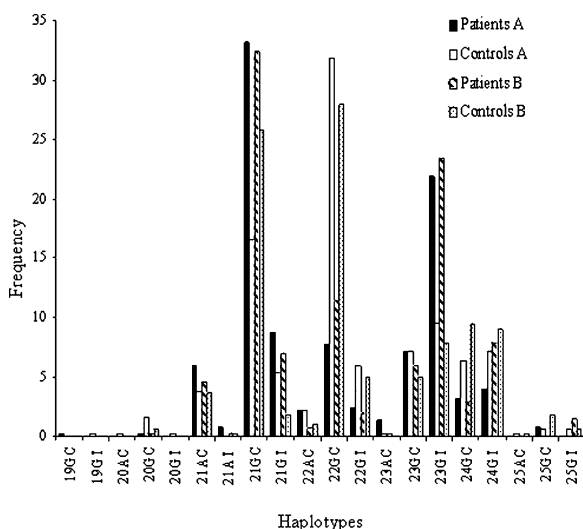


FIG 2. Frequency distribution of the haplotypes of the *TGFβ1* gene in patients and unrelated controls in cohorts A and B, respectively. The haplotypes were plotted on the x-axis and their respective relative frequencies (%) on the y-axis.

test the effect of these polymorphisms and of disease status on total serum IgE levels and serum TGF-β1 levels. Multiple logistic regression analysis was performed to determine the effect of age and sex, if any, on the association of *TGFβ1* genotypes with asthma.

Haplotypes for each individual were inferred by using the algorithm developed by Stephens et al¹⁸ (PHASE), which uses a Bayesian approach incorporating a priori expectations of haplotypic structure from population genetic and coalescent theory. Default parameters were used to generate these haplotypes, and the differences in frequencies were compared in the 2 groups. Individuals with phase probabilities less than 80% were excluded in the analysis. ORs were calculated for haplotypes whose distribution was significantly different in the 2 groups studied.

RESULTS

To explore the association of SNPs and repetitive sequences in and around the *TGFβ1* gene with asthma, we genotyped patients and controls in 2 independent cohorts, as described in Methods.

Association of *TGFβ1* polymorphisms with asthma

In initial genotyping of TGF-β1 polymorphisms, 2 SNPs (–988C/A and 788C/T) reported to be polymorphic in other populations were found to be nonpolymorphic in the Indian population (N = 200).

We identified a novel (CT)_n(CA)_m repeat polymorphism 24.9 kb upstream of the *TGFβ1* gene (Fig 1) and used it for genotyping patients and controls in 2 independent cohorts. A total of 7 alleles were obtained for cohort A (heterozygosity index = 0.728) and 6 alleles for cohort B (heterozygosity index = 0.730; Table III). A highly significant difference was obtained in the allele count in the patients and the controls (KS χ^2 = 34.22; *df* = 6; *P* = .00001 in cohort A; and KS χ^2 = 11.29; *df* = 5; *P* = .003 in cohort B). The distribution was statistically significant after Bonferroni correction (^A α = 0.007; ^B β = 0.008) in both the cohorts.

For the –800G/A and the –509C/T polymorphisms, the allele and genotype frequencies are depicted in Table IV. The distributions of genotype frequencies of these 2 SNPs were consistent with Hardy-Weinberg expectations in the controls for both the cohorts. At the level of genotypes, the AA homozygous was rare for the –800G/A SNP, with only 1 individual in the controls. By using multiple logistic regression analyses, we confirmed the association between the (CT)_n(CA)_m, –800G/A, and –509C/T polymorphisms with asthma, and it was found not to be influenced by sex and age (Table V).

Association of *TGFβ1* polymorphisms with serum IgE

Total serum IgE levels were found to follow a log normal distribution in both the cohorts. When the cases and controls were compared with respect to the log total serum IgE levels, a highly significant difference was obtained (for cohort A, *t* test = 3.58; *df* = 255; *P* < .0004; and for cohort B, *t* test = 6.58; *df* = 285; *P* < .0004).

Because increased total serum IgE level is one of the major characteristics of atopic asthma and TGF-β1 is known to regulate IgE,⁶ the genetic effects of *TGFβ1*

polymorphisms were tested on serum IgE levels. A significant effect was observed at the level of alleles for the (CT)_n(CA)_m repeat ($P = .0039$ and $P = .0001$ for cohorts A and B, respectively). Similar results were obtained at the level of genotypes ($P = .0315$ and $P = .0002$ for cohorts A and B, respectively).

At the level of alleles, a significant effect with serum IgE levels was observed for $-509C/T$ SNP ($P = .003$ and $P = .02$ for cohorts A and B, respectively). A significant effect was also seen at the level of genotype for $-509C/T$ polymorphism ($P = .006$ and $P = .019$ for cohorts A and B, respectively). However, no significant difference with respect to log total serum IgE was observed for the $-800G/A$ polymorphism at the level of alleles or genotype.

Association of *TGFβ1* haplotypes with asthma and total serum IgE levels

The 20 haplotypes in the case of cohort A and 16 in the case of cohort B were generated by combinations of the (CT)_n(CA)_m repeat and 2 SNPs ($-509C/T$ and $-800G/A$) by using PHASE (Fig 2).

The haplotypes 21_G_C and 23_G_T were the most frequent in the patient population. The odds of patients rather than controls having 21_G_C was 2.45, with 99% CI = 1.58 to 3.94 (likelihood $\chi^2 = 27.92$; $df = 1$; $P = .00001$) and 1.38 with 95% CI = 1.01 to 1.87 (likelihood $\chi^2 = 4.21$; $df = 1$; $P = .04$) for cohorts A and B, respectively. Similarly, the odds of patients rather than controls having 23_G_T was 2.64 with 99% CI = 1.51 to 4.59 (likelihood $\chi^2 = 21.77$; $df = 1$; $P = .00001$) and 3.55 with 99% CI = 2.00 to 6.29 (likelihood $\chi^2 = 37.22$; $df = 1$; $P = .00001$) for cohorts A and B, respectively. These results suggest that 21_G_C (in cohort A) and 23_G_T (in both cohorts) are risk/susceptibility haplotypes and are positively associated with asthma.

On the other hand, the haplotypes 22_G_C and 24_G_C were the most frequent in the control population. The odds (OR) of patients rather than controls having 22_G_C was 0.18 with 99% CI = 0.10 to 0.31 (likelihood $\chi^2 = 35.32$; $df = 1$; $P = .00001$) and 0.33 with 99% CI = 0.20 to 0.54 (likelihood $\chi^2 = 72.28$; $df = 1$; $P = .00001$) for cohorts A and B, respectively. Similarly, the odds of patients rather than controls having 24_G_C was 0.48 with 95% CI = 0.24 to 0.98 (likelihood $\chi^2 = 4.28$; $df = 1$; $P = .038$) and 0.28 with 99% CI = 0.12 to 0.68 (likelihood $\chi^2 = 16.01$; $df = 1$; $P = .00001$) for cohorts A and B, respectively. These results suggest that 22_G_C (in both cohorts) and 24_G_C (in cohort B) are protective haplotypes and are negatively associated with asthma.

On comparing the different haplotypic combinations with respect to the log total serum IgE levels, a highly significant difference was obtained (for cohort A, F ratio = 1.92; $df = 18$; $P = .012$; and for cohort B, F ratio = 2.83; $df = 15$; $P = .0003$).

Functional correlation of *TGFβ1* gene polymorphisms with serum levels of TGF-β1

To find any functional correlation of *TGFβ1* gene polymorphisms with its expression in serum, the concen-

TABLE VI. Serum TGF-β1 levels in context of alleles, genotypes, and haplotypes

(CT) _n (CA) _m alleles	Number	Log TGF-β1 levels (±SE)	F ratio	df	P value
21	50	1.73 (±0.03)	4.59	3	.004
22	33	1.59 (±0.04)			
23	37	1.76 (±0.04)			
24	15	1.62 (±0.06)			
–509 C/T genotype					
CC	28	1.62 (±0.06)			
CT	46	1.79 (±0.05)	9.63	2	.0002
TT	7	2.24 (±0.13)			
Haplotype					
22_G_C/22_G_C	5	1.41 (±0.13)			
22_G_C/Non22_G_C	20	1.61 (±0.07)	6.87	2	.0019
Non22_G_C/Non22_G_C	47	1.83 (±0.04)			
23_G_T/23_G_T	3	2.09 (±0.15)			
23_G_T/Non23_G_T	23	1.79 (±0.05)	4.77	2	.01
Non23_G_T/Non23_G_T	46	1.66 (±0.04)			

tration of TGF-β1 in sera samples of 81 unrelated individuals was determined by using ELISA. The levels of TGF-β1 with different (CT)_n(CA)_m repeat alleles were found to be significantly different (Table VI). Particularly, alleles 22 and 24 were associated with low TGF-β1 levels, whereas alleles 21 and 23 were associated with higher levels. The $-509C/T$ polymorphism was also found to affect the serum levels of TGF-β1. As shown in Table VI, the presence of T allele is associated with higher concentrations of TGF-β1, and this effect seems to be dose-dependent, because the CT heterozygotes had lower levels compared with the TT homozygotes.

When the haplotypes were analyzed for TGF-β1 levels, a significant difference was observed (F ratio = 2.82; $df = 11$; $P = .0024$). As seen from the Table VI, individuals with the protective haplotype 22_G_C had lower TGF-β1 levels compared with individuals with the positively associated haplotype 23_G_T.

DISCUSSION

TGF-β1 is produced by nearly all cell types of the immune system and is an important immunomodulatory molecule. The predominant functions of TGF-β1 are anti-inflammatory, as shown in various models of airway inflammation and airway hyperresponsiveness.^{19–21} However, the secretion of TGF-β1 immediately after an allergic disorder contributes to fibrosis and the irreversible changes associated with airway remodeling in chronic asthma, thus pointing toward a proinflammatory role for this cytokine.²²

To elucidate the role of TGF-β1 in asthma pathogenesis, we identified and genotyped a novel repeat (BV209662) and also screened known polymorphisms: $-988C/A$, $-800G/A$, $-509C/T$, and $788C/T$ (Fig 1). Two other polymorphisms, 869T/C and 915G/C, were not genotyped in this study, because earlier studies reported

a strong LD among $-509C/T$, $869T/C$, and $915G/C$.^{10,11} Also, the $-509C/T$ polymorphism plays a greater role in asthma, as seen in a recent study by Silverman et al,²³ which showed that the C to T transition at position -509 augments Yin-Yang 1 transcription factor binding and enhances basal promoter function for *TGF β 1* gene; the T allele is thus associated with higher levels of TGF- β 1 transcript. Nevertheless, it would be interesting to investigate the $869T/C$ and $915G/C$ SNPs in our population to determine their LD pattern with $-509C/T$.

In accordance with the study in the Caucasian population,¹¹ we have found the association of the $-509C/T$ polymorphism with asthma and serum IgE. In our study population, the CC genotype was overrepresented in the control group, and on being changed to CT, a significant increase in risk was observed (Table IV). We also observed that the individuals with CC genotype had the lowest TGF- β 1 levels in their serum, followed by CT, whereas the TT homozygotes had the highest levels (Table VI). Our observations support the study conducted by Silverman et al.²³ In contrast, a study conducted in Czech population did not find any association for this SNP with atopy-associated quantitative traits such as total serum IgE levels.¹⁴ This could be a result of differences in phenotype definitions, ethnicity, and environmental exposure to allergens.

Earlier studies indicate that dinucleotide repeats located far upstream of the promoter are involved in gene regulation.²⁴⁻²⁶ For example, a far upstream (CA)_n repeat (allele 25) was found to repress Ha-ras gene activity.²⁶ In our study, the alleles of the (CT)_n(CA)_m locus showed a significant difference in distribution pattern between the patient and control groups (Table III). Interestingly, the different alleles were found to be associated with different levels of TGF- β 1 (Table VI). However, the functional role of these repeats is yet to be established.

To further the understanding of the contributions of this gene toward asthma, we constructed 3-locus haplotypes by using PHASE.¹⁸ The overall distribution of the different haplotypic combinations was significantly different in the patients and the controls. In addition, we identified 2 major haplotypes, 22_G_C and 24_G_C, as protective haplotypes for asthma. In contrast, the haplotypes 21_G_C and 23_G_T increase the susceptibility of the patient to develop this disorder, marking them as the major risk haplotypes. As already validated for the (CT)_n(CA)_m and -509 loci individually, the protective haplotypes were associated with lower serum TGF- β 1 levels and vice versa. Particularly, the individuals homozygous for the 22_G_C haplotype have the lowest levels of TGF- β 1, followed by heterozygous individuals, whereas the ones lacking this haplotype have the highest levels of TGF- β 1 in their sera (Table VI). This dose dependency is also reflected in the individuals with 23_G_T, wherein individuals homozygous for this haplotype have the highest levels of TGF- β 1 in their sera. Thus, the polymorphisms constituting the 3-locus haplotypes may be controlling TGF- β 1 levels directly or may be present in LD with another true functional poly-

morphism within and/or around the gene. Although the individuals homozygous for either the 22_G_C or the 23_G_T haplotypes were few ($n = 5$ and $n = 3$, respectively), the difference in TGF- β 1 levels with each haplotype was highly significant ($P = .0019$ in the case of 22_G_C and $P = .01$ for the 23_G_T haplotype). Appreciable numbers of individuals heterozygous for either haplotype have corroborated this observation further. However, to gain insight into tissue-specific expression of TGF- β 1, these results must be further validated by using bronchoalveolar lavage fluid/lung biopsy samples.

Because asthma is a complex disorder, we conducted a case-control study that could provide better directions on promising loci.¹⁷ These leads can be further tested by using a well-controlled family-based study. Indeed, we have established the genetic homogeneity between the 2 groups by genotyping multiple loci to eliminate the errors caused by stratification or an inherent statistical bias.¹⁷ Moreover, the additional unlinked regions used as controls²⁷ and validation of our results in another independent cohort could only add more confidence to our results.

In summary, this is the first study in any population identifying novel protective and risk haplotypes of the *TGF β 1* gene. These haplotypic combinations have further been functionally validated. In addition to supporting the already known association of $-509C/T$ polymorphism with the TGF- β 1 levels, we report here the association of the (CT)_n(CA)_m repeat alleles with the TGF- β 1 levels. In contrast with earlier observations, in which TGF- β 1 was primarily implicated to mediate anti-inflammatory actions, in our study, low TGF- β 1 levels associated with protective genotypes as well as haplotypes point toward the proinflammatory role of this cytokine. During the development of an allergic reaction, TGF- β 1 is a negative regulator of inflammation, but in the case of chronic asthma (as in the patient population we studied), in which the inflammatory conditions are already established, TGF- β 1 exacerbates lung deterioration and thus has a proinflammatory role.

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