TGFβ1 haplotypes and asthma in Indian populations

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Background: Asthma is a complex disorder of the airways of the lungs. TGF-β1 plays a key role in airway remodeling and asthma by having both proinflammatory and anti-inflammatory activities, making *TGFβ1* an important candidate gene to study.
Objective: To investigate the association of *TGFβ1* 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-β1 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 *TGFβ1* 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-β1 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-β1 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 *TGFβ1* gene that are associated with asthma. We also demonstrate the functional significance of these haplotypes with serum TGF-β1 levels. These results would be valuable in elucidating the role of TGF-β1 in asthma pathogenesis.
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Key words: *Asthma, case-control study, haplotype, Indian population, serum IgE, TGFβ1*

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

TGF-β1 plays an important role in airway remodeling, an established pathological feature in asthma.[1,2] It is implicated in several aspects of fibrosis[3] wherein subepithelial 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-β1 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 *TGFβ1* a promising candidate gene for asthma.

The *TGFβ1* gene is located on chromosome 19q13.1-13.2[8] 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 levels[12] and TGF-β1 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 *TGFβ1* 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 ± 0.07	2.40 ± 0.05	2.85 ± 0.07	2.87 ± 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.

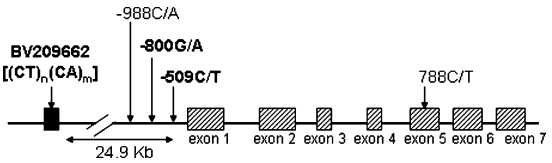

FIG 1. Gene structure and polymorphisms investigated in the *TGFβ1* gene. The positions marked in *bold* were found polymorphic in the Indian population.

(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-β1 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 ± 0.97 years and 34.22 years ± 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) guidelines[15] 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-β1 level measurement

We used the TGF-β1 ELISA system (R&D Systems, Minneapolis, Minn) as per the manufacturer's instructions to determine total TGF-β1 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 described[16] 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,