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ORIGINAL ARTICLE

Genetic variation of *TBX21* gene increases risk of asthma and its severity in Indian children

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T-box transcription factor protein (TBX21) is encoded by the *TBX21* gene in human. It is crucial for naive T lymphocyte development, interferon-γ production, airway hyperresponsiveness and regulation of corticosteroid response in asthmatics. Polymorphisms rs4794067 and rs16947078 of *TBX21* were found to be associated with acetylsalicylic acid-induced and allergic asthma, respectively. We examined whether sequence variants of *TBX21* gene are associated with asthma and its severity in Indian population. In a hospital-based case–control study, 240 asthmatic children and 240 healthy controls were investigated for the association of *TBX21* rs4794067 (C > T) and rs16947078 (G > A) polymorphisms with asthma and its severity using PCR-restriction fragment length polymorphism method. Heterozygous (CT) (odds ratio (OR) = 2.33; *P* = 0.001) and variant (TT) (OR = 6.25; *P* = 0.001) genotypes of rs4794067 were demonstrated significant risk of asthma. However, in asthma severity variant (TT) genotype revealed significant increase risk (intermittent: OR = 5.9, *P* = 0.001; mild: OR = 8.0, *P* = 0.001; moderate: OR = 3.2, *P* = 0.041; and severe: OR = 43.6, *P* = 0.001) in all subgroups. Furthermore, haplotypes TG (OR = 2.83; *P* = 0.001) and TA (OR = 2.54; *P* = 0.001) of *TBX21* were associated with an increased risk of asthma. Conversely, rs16947078 G > A polymorphism was not associated with any asthma/asthma severity risk. These data suggest that *TBX21* gene variation may modify individual's susceptibility to asthma and its severity in Indian population. However, further validation in large population-based studies is needed to confirm the finding.
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INTRODUCTION

Asthma is a common and combinatorial disorder with genetic, environmental and lifestyle determinants.[1] It is prevalent in developed nations and becoming an important health issue in many developing countries. It is likewise a major cause of the socioeconomic burden on the health-care system and poor quality of life for sufferers. Global estimates indicated that 300 million peoples are affected with asthma, and due to urbanization this number would increase to 400 million by 2025.[2,3] In India, recent report showed wide variation (4–19%) in the prevalence of asthma. There was a low prevalence of asthma (2.3–3.3%) in the children surveyed in Lucknow, North India.[4]

The etiology of asthma is not yet clear. The immunohistopathologic features include Th2-mediated acute inflammation, elevated serum immunoglobulin-E level, increased number of eosinophils, mucus secretion and airways smooth muscles thickening.[5] It has been reported that the balance between Th1 and Th2 cytokines orchestrate the inflammation of the lung and the progression of asthma.[5,6] Regulatory molecules such as sexual factors, chemokines, signal-transduction pathway (for example, Stat6 for Th2 development and Stat4 for Th1 development), transcription factors (TBX21 and GATA-3) and T-helper cells play directive role to maintain the balance of Th1/Th2 immune responses.[7–9] Lately, the transcription factor TBX21 has been placed as a central regulator of type 1 immunity and thought to be necessary for balance of the Th1/Th2 immune system.[5] *In-vivo* and *in-vitro* experiments demonstrated the effect of TBX21 in Th2 committed cells and activation of interferon-γ production in CD4 T-helper cells.[5,9] The high expression of TBX21 in Th1 cells induce Th1 cytokines (interferon-γ and interleukin-12 (IL-12)) and suppress Th2 cytokine (IL-4 and IL-5) production. An imbalance between Th1 and Th2 cytokines due to decrease in Th1 response is the main cause of inflammation and development of asthma.[5,9,10] Markedly decreased expression of the TBX21 in $CD4^+$ T cell in asthmatics suggested that the loss of TBX21 might be associated with asthma and its severity. Linkage and twins study showed that T-bet expression, interferon-γ production and Th2-associated diseases are under strong genetic influence and might be due to genetic variations in Th1 cytokine regulation via *TBX21*.[11–16]

These observations are indicating the importance of transcription factor TBX21 in asthma pathobiology. Recently, the molecular epidemiological studies have suggested the effect of *TBX21* gene variants on asthma risk. However, results of genetic association

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studies differ with ethnicity.[17] Studies from Finland and Korea demonstrated no association between *TBX21* gene polymorphisms and asthma.[18,19] Whereas, polymorphisms rs9910408, rs35120858, rs59544687 and rs2325717 were found to be significantly associated with airways hyperresponsiveness in children with asthma in a North American clinical trial.[20] Similarly, a most common promoter rs4794067 (T1993C) polymorphism was found to be significantly associated with acetylsalicylic acid-induced asthma in Japanese.[21] *In-vitro* functional experiments also demonstrated that the *TBX21* T1993C polymorphism repress TBX21 expression and Th1 cytokine production through control of Yin Yang 1 (YY1) transcription factors.[22] Likewise, in another study, rs16947078 polymorphism was found to be significantly associated with allergic asthma.[23]

In light of the strong support for the involvement of *TBX21* in asthma, the present study was designed to evaluate the association between *TBX21* (rs4794067 and rs16947078) polymorphisms and asthma/severity in North Indian children. To the best of our knowledge, no study has been reported from India on the association of these polymorphisms with the risk of asthma.

MATERIALS AND METHODS

Study type and setting

This was a hospital-based case–control study conducted at the Department of Paediatrics, King George's Medical University, Lucknow, Uttar Pradesh, between September 2010 and July 2013. This study was approved by the institutional ethics committee (letter no. 2824/R-cell-11), and written informed consent was obtained from the parent/guardian of all participants. A questionnaire was filled by parent/guardian on providing information of ethnicity, education, religion, socioeconomic status, family characteristics, residential environment, medication, asthma history of subject and their family and so on.

Subjects

We have recruited 480 subjects (240 asthmatic and 240 controls) of same ethnicity. In screening of asthma, patient had at least one of the following symptoms positive: (i) current presence of wheeze with a history of more than one episode of documented wheeze or use of bronchodilator in the preceding 12 months or (ii) first episode of wheeze with positive family history of asthma in parents or sibling. We have excluded the subjects who had pneumonia, tuberculosis, disseminated bronchiectasis, bronchiolitis, pnemothorax, pyothorax, immunocompromised status, malignancy and above 15 years age. The inclusion criteria for control subjects were as follows: (i) 1–15 years of age, no present symptoms or history of asthma or other respiratory disease (ii) no history of atopy and (iii) without family history of asthma in mother, father or sibling. To make a diagnosis of asthma and severity we used NIH guideline-2007 and categorized them in four subgroups.[24]

Lung function

Spirometry test was done in children above 6 years of age using fully computerized portable Spirometer (Spiro lab II, MIT II, Longfian Scitech Company, Boading, China) and forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio were measured. The FEV1/FVC ratio higher than 0.9 was defined normal (GINA: assessable at https://www.google.co.in/#q=gina+guidelines).[2]

Genomic DNA extraction and genotyping of *TBX21* variants

Blood samples (3.0 ml) from asthma patients and control subjects were collected in EDTA vials and stored at −20 °C until required. Genomic DNA was extracted from peripheral blood using salting-out method.[25] Genotyping was performed using PCR-restriction fragment length polymorphism. For rs4794067 polymorphism (C>T), PCR was performed using published primers set.[26] A primer designing tool batch primer3 (accessible at http://probes.pw.usda.gov/cgi-bin/batchprimer3/batchprimer3.cgi) was used for designing primers in such a way that it can amplify DNA containing the rs16947078 (G>A) polymorphism.[27] The forward and reverse primers were 5′-ACGGTGGCATACTTGGCTCCCTT-3′ and 5′-ACCACATCAAAACA TGCAGTCAGAC-3′, respectively. NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and UCSC *in-silico* PCR (http://genome-mirror.duhs.duke.edu/cgi-bin/hgPcr) were used to verify the sequences of designed primer.[28,29] Each PCR was performed in a total reaction volume of 10 µl, with 30 ng of genomic DNA, 1 µl 10× buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.5U Taq polymerase (New England Biolabs, Ipswich, MA, USA) and 1 pmol of each primer. PCR conditions were as follows: initial denaturation of 95 °C for 2 min, 35 cycles of 94 °C for 1 min, included an annealing temperature 62 °C/45s and 64 °C/60s for the polymorphisms rs4794067 and rs16947078, respectively. Five microliters of PCR products were digested overnight with 1 unit of restriction enzyme (*Hha*I, New England Biolabs, for C>T variant and *Hph*I, New England Biolabs, for G>A variant) at 37 °C. The expected sizes of specific genotype were seen under UV light in agarose gel stained with ethidium bromide; Figures 1a and b. Positive and negative controls were used in each genotyping assay and 5% of randomly selected samples were re-genotyped by other lab personal with 100% concordance.

Sequencing

For quality control of PCR-restriction fragment length polymorphism genotyping results, we randomly selected 10% of the samples for sequencing, and gained 100% reproducibility (Figures 1c and d).

Statistical analysis

The collected data were analyzed using EpiNfo6 (available from the centers for disease control and prevention: Atlanta, GA, USA; http://www.cdc.gov/epi/epiinfo.htm), INSTAT 3.0 and SPSS15 (Chicago, IL, USA) statistical tools. χ^2-Test was used to determine deviation from Hardy–Weinberg equilibrium and difference in genotype/allele frequency.[30] For continuous measures, descriptive statistics were presented as mean and standard deviations compared by the Student's *t*-test. Fisher's exact test was carried out to avoid type 1 error in the subgroup analysis. Odds ratios (ORs) were determined by logistic regression and adjusted for age, sex and other covariates. Most frequent genotype was used as reference, and statistical significant was determined at $P<0.05$ for all tests. SNPAnalyzer Ver1.0 was used to analyze linkage disequilibrium between polymorphisms and to determine haplotype frequencies.[31] Power analysis was performed by CaTS—power calculator with settings of multiplicative genetic model.[32]

RESULTS

Demographic details

The case group consists of 240 asthmatic children, including 33.3% female subjects. Among the patients, 85 (35.41%) were intermittent, 86 (35.83%) and 47 (19.58%) were mild and moderate persistent, and 22 (9.16%) was severe persistent. Table 1 represents the demographic characteristics of the studied subjects. Among the case and control groups, cases were at risk if father had a smoking habit ($P=0.001$). Similarly, residence near vehicular traffic, proximity to an industrial area and urbanization were the factors likely to cause a risk in development of asthma ($P\leqslant0.05$).

Association of *TBX21* gene variants with asthma compared with controls

The genotype and allele frequency distribution in patients and healthy controls are shown in Table 2. The distribution of observed genotypes frequency in the healthy controls did not deviate from Hardy–Weinberg equilibrium. The association between *TBX21* gene polymorphisms rs4794067 and rs16947078 were analyzed by logistic regression. Increase risk for asthma was observed in heterozygous CT (OR = 2.33, 95% confidence interval (CI) 1.28–4.24, $P=0.001$) and homozygous TT (OR = 6.25, 95% CI 3.37–11.59, $P=0.001$) genotypes of *TBX21* rs4794067 polymorphism. Similarly, variant allele carrier (CT + TT) also demonstrated threefold increased risk for asthma (OR = 3.70, 95%

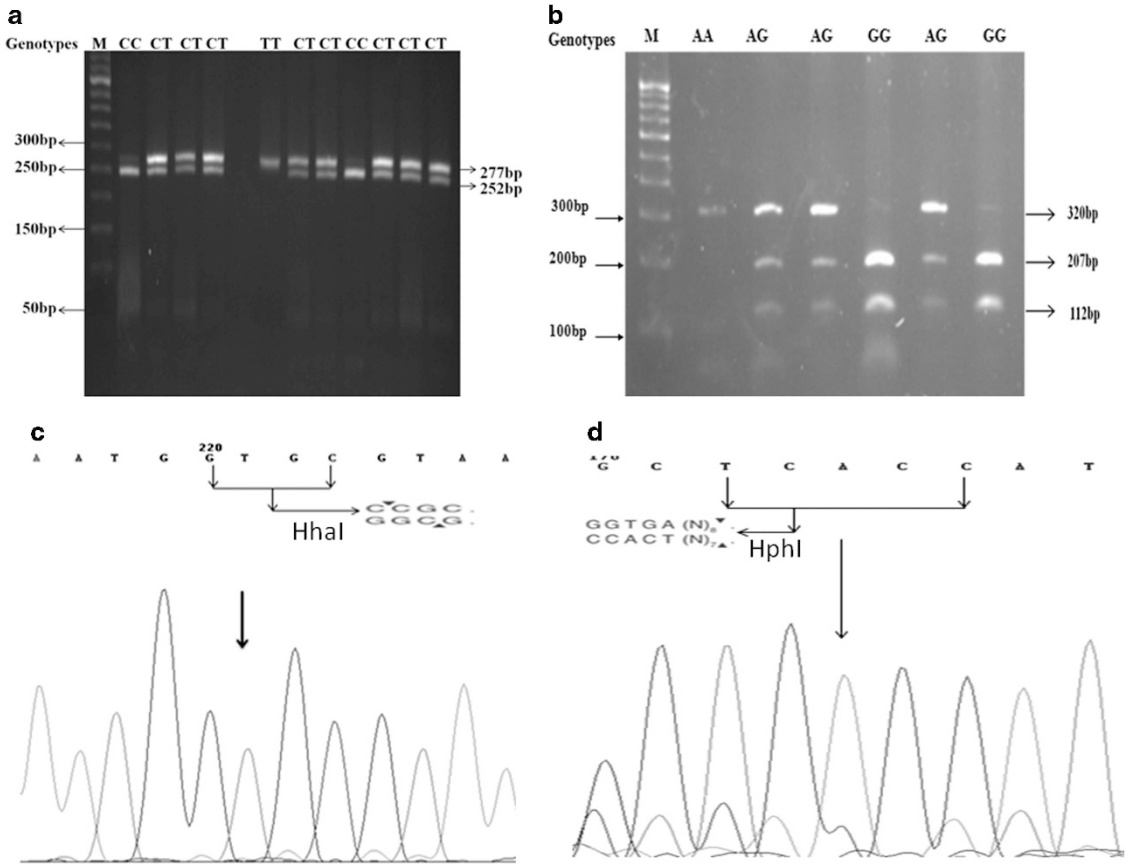

Figure 1 Genotyping of *TBX21* gene variants by PCR-restriction fragment length polymorphism and validation of results through sequencing. (**a**) Genotyping of *TBX21* (rs4794067: C>T) gene variant. An agarose gel showing M = 50 base-pair molecular marker, Genotypes and size of digested PCR products: CC = 252 base pair + 25 base pair, CT = 277 base pair + 252 base pair + 25 base pair, TT = 277 base pair. Twenty five base pair-digested PCR products were pass out through 1.8% agarose gel, therefore it is not visible in agarose gel. This genotyping was performed by restriction enzyme digestion with *Hha*I. (**b**) Genotyping of *TBX21* (rs16947078: G>A) gene variant. A two percent agarose gel showing M = 100 base-pair molecular marker, genotypes and size of digested PCR products: AA = 320 base pair, AG = 112 base pair + 207 base pair + 320 base pair, GG = 112 base pair + 207 base pair. This genotyping was performed by restriction enzyme digestion with *Hph*I. (**c**) A sample chromogram showing the presence of 'T' allele of the rs4794067; C>T SNP. (**d**) A sample chromogram showing the presence of 'A' allele of the rs16947078; G>A SNP. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

CI 2.11–6.49, $P = 0.001$). However, no significant association was found between polymorphism rs16947078 and asthma.

Power analysis of *TBX21* rs4794067 polymorphism revealed 88% power to detect a significant effect with prevalence of 3.3% disease, disease allele frequency 49.6%, genotype relative risk 1.5 and significance level 0.05. However, rs16947078 polymorphism showed 72% power with disease allele frequency 17.1%, genotype relative risk 1.5 and 0.05 of significance level.

Genetic susceptibility of *TBX21* gene variants with severity of asthma

The genotype frequencies among subgroups of cases are shown in Table 3. Subsequent analysis pertaining to the assessment of risk associated with subgroups of asthma and healthy subjects depicted significant association with variant genotype (rs4794067: TT) in intermittent (OR = 5.94, 95% CI 2.50–14.14; $P = 0.001$), mild persistent (OR = 8.0, 95% CI 3.04–21.07; $P = 0.001$), moderate persistent (OR = 3.22, 95% CI 1.23–8.45; $P = 0.041$) and severe persistent (OR = 43.62, 95% CI 3.06–621.48; $P = 0.001$) subgroups. Similarly, significant association was also observed for CT + TT in intermittent (OR = 3.22, 95% CI 1.44–7.20; $P = 0.002$) mild persistent (OR 5.12, 95% CI 2.05–12.78; $P = 0.001$) and severe persistent (OR = 13.25; 95% 1.20–146.35; $P = 0.004$) subgroups. We found that CT + TT was marginally associated with moderate persistent (OR 2.26, 95% CI 0.94–5.42; $P = 0.051$) subgroup. However, we did not find any significant association between severity and rs16947078 polymorphism.

Association of *TBX21* gene variants with family history of asthma risk

A case-only analysis was performed to find out possible association between genotype of asthmatic children with and without family history of asthma (Table 4). We did not find any significant association with both groups for *TBX21* variants rs16947078 and rs4794067.

Association of *TBX21* haplotype with asthma

Further, to elucidate the combined influence of both polymorphisms, we constructed haplotype of *TBX21* gene polymorphisms (Table 5). The ORs were estimated with reference to haplotype consisting of wild-type alleles (C–G). Haplotypes with a frequency of <1% were excluded for statistical analysis. The frequency of CG haplotype was more in the control group (0.41) than in the asthmatic (0.21).

Table 1 Basic profile and residential environment of studied subjects from North Indian population

Subject's characteristics	*Cases* (n = *240)*	*Controls* (n = *240)*	P-*value*
Sex, female (*n*%)	80 (33.3)	83 (34.6)	0.847
Age in month (mean ± s.d.)	71.01 ± 40.315	76.46 ± 38.154	0.104
Weight in kg (mean ± s.d.)	18.02 ± 9.522	17.82 ± 7.53	0.807
Height in cm (mean ± s.d.)	107.92 ± 21.42	107.59 ± 21.926	0.867

Residential environment	*Cases* (n = *240)*	*Controls* (n = *240)*	*OR (95% CI)*	P-*value*
Urban (*n*%)	135 (56.3)	98 (40.8)		
Rural (*n*%)	105 (43.8)	142 (59.2)	1.9 (1.29–2.67)	**0.001**
Animals in house (*n*%)	85 (35.4)	79 (32.9)	1.1 (0.76–1.63)	0.630
Use of biomass as fuel (*n*%) (other than LPG for cooking)	115 (47.9)	81 (33.0)	1.8 (1.24–2.61)	**0.002**
Industry/factory (*n*%)	36 (15.0)	12 (5.0)	3.35 (1.69–6.62)	**0.001**
Heavy traffic in km (mean ± s.d.)	1.22 ± 3.32	1.65 ± 1.76	—	0.077
Type of road				
With occasional traffic (*n*%)	49 (20.4)	110 (45.8)	0.30 (0.20–0.45)	**0.001**
With vehicular traffic (*n*%)	183 (76.3)	127 (52.9)	2.85 (1.93–4.22)	**0.001**
Near heavy traffic (*n*%)	8 (3.3)	3 (1.3)	2.72 (0.71–10.39)	0.222
Smoking by father (*n*%) (current or ex-smoker)	92 (33.5)	52 (21.7)	2.24 (1.50–3.36)	**0.001**

Abbreviations: CI, confidence interval; cm, centimeter; kg, kilogram; km, kilometer; LPG, liquid petroleum gas; OR, odds ratio.
Significant *P*-values ⩽ 0.05 are shown in bold.

Table 2 Distribution of *TBX21* gene variants among asthmatic (*n* = 240) and control subjects (*n* = 240)

Genotypes	*Controls,* n *(%)*	*Cases,* n *(%)*	*OR (95% CI)*	P-*value*	*OR (95% CI) Adjusted for covariates*[a]	P-*value Adjusted for covariates*[a]
TBX21 (db SNP ID rs4794067; C>T)						
CC	64 (26.7)	23 (9.6)	1 (Reference)		1 (Reference)	
CT	114 (47.5)	85 (35.4)	2.0 (1.19–3.60)	**0.010**	2.33 (1.28–4.24)	**0.001**
TT (codominant model)	62 (25.8)	132 (55.0)	5.9 (3.37–10.41)	**0.001**	6.25 (3.37–11.59)	**0.001**
CT + TT (dominant model)	176 (73.33)	217 (90.40)	3.4 (2.04–5.75)	**0.001**	3.70 (2.11–6.49)	**0.001**
CT + CC versus TT (recessive model)	178 (74.16)	108 (45.00)	3.5 (2.39–5.16)	**0.001**	3.41 (2.23–5.21)	**0.001**
CC + TT versus CT (over-dominant model)	126 (52.50)	155 (64.60)	0.6 (0.42–0.41)	**0.007**	0.67 (0.45–1.00)	**0.048**
Allele frequency						
C (multiplicative model)	242 (50.4)	130 (27.4)	1 (Reference)		1 (Reference)	
T (multiplicative model)	238 (49.6)	350 (72.6)	2.5 (1.92–3.31)	**0.001**	2.5 (1.88–3.41)	**0.001**
TBX21 (db SNP ID rs16947078; G>A)						
GG	167 (69.6)	169 (70.4)	1 (Reference)			
AG	64 (26.7)	59 (24.6)	0.9 (0.60–1.37)	0.658	1.04 (0.66–1.65)	0.686
AA (codominant model)	9 (03.8)	12 (5.0)	1.3 (0.54–3.20)	0.544	1.26 (0.48–3.29)	0.890
AG + AA (dominant model)	73 (30.41)	71(29.59)	0.9 (0.65–1.42)	0.920	1.07 (0.70–1.65)	0.750
AG + GG versus AA (recessive model)	231 (96.25)	228 (95.00)	1.3 (0.56–3.27)	0.500	1.24 (0.48–3.23)	0.660
GG + AA versus AG (over-dominant model)	176 (73.33)	181 (75.40)	0.9 (0.59–1.35)	0.675	1.03 (0.65–1.62)	0.900
Allele frequency						
G (multiplicative model)	398 (82.9)	396 (82.6)	1 (Reference)		1 (Reference)	
A (multiplicative model)	82 (17.1)	84 (17.4)	1.0 (0.73–1.43)	0.932	1.08 (0.76–1.53)	0.670

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]OR and *P*-value were adjusted for covariates (age, sex, residential environment and smoking habit of father).
Significant *P*-values ⩽ 0.05 are shown in bold.

However, the frequency of haplotypes TG and TA were significantly higher in patients and showed increased risk. The pair-wise linkage disequilibrium analysis in cases ($D = 0.0119$; $P = 0.150$) and control ($D = -0.0006$; $P = 0.956$) did not show any linkage between both polymorphisms. The haplotye interaction analysis with covariate sex also showed significantly higher risk in male for TG and TA haplotypes, while females with TG haplotype were at risk for asthma.

Risk of asthma associated with SNPs stratified by environment (Father's smoking habits and industry/factory nearby residence)

Case–control analyses (between genotypes and smoking habits of father, factory or industry nearby residence) were performed to evaluate possible gene–environment interactions (Table 4). We observed that the presence of homozygous variant (TT) significantly increased the risk of asthma in both the groups: father with

Table 3 Genetic susceptibility of the *TBX21* gene variations with severity of asthma

Genotypes	*Controls* (N = *240*)	*Intermittent,* N = *85 (OR 95%; P-value)*	*Mild persistent,* N = *86 (OR 95%; P-value)*	*Moderate persistent,* N = *47 (OR 95%; P-value)*	*Severe persistent,* N = *22 (OR 95%; P-value)*
TBX21 (db SNP ID rs4794067; C>T)					
CC	64	9 (Reference)	6 (Reference)	7 (Reference)	1 (Reference)
CT	114	27 (1.6; 0.72–3.69; 0.240)	33 (3.00; 1.21–7.69; 0.018)	21 (1.6; 0.66–4.11; 0.281)	4 (2.0; 0.21–18.70; 0.534)
TT (codominant model)[a]	62	49 (5.94; 2.50–14.14; 0.001)	47 (8.01; 3.04–21.07; 0.001)	19 (3.22; 1.23–8.45; 0.041)	17 (43.62; 3.06–621.48; 0.001)
CC Vs CT + TT (dominant model)[a]	176	76 (3.22; 1.44–7.20; 0.002)	80 (5.12; 2.05–12.78; 0.001)	40 (2.26;0.94–5.42; 0.051)	21 (13.25; 1.20–146.35; 0.004)
CT + CC Vs TT (recessive model)[a]	178	36(3.93; 2.19–7.04; 0.001)	39 (3.10; 1.77–5.43; 0.001)	28 (2.16; 1.10–4.24; 0.028)	5 (14.84; 4.12–53.50; 0.001)
CC + TT Vs CT (over-dominant model)[a]	126	58(0.54; 0.31–0.96; 0.034)	53 (0.82; 0.48–1.40; 0.460)	26 (0.88; 0.46–1.67; 0.690)	18 (0.22; 0.07–0.77; 0.008)
TBX21 (db SNP ID rs16947078; (G>A)					
GG	167	62 (Reference)	60 (Reference)	27 (Reference)	20 (Reference)
AG	64	19 (0.8; 0.45–1.50; 0.536)	21 (0.9; 0.50–1.61; 0.737)	18 (1.7; 0.88–3.34; 0.108)	1 (0.1; 0.02–1.00; 0.050)
AA (codominant model)[a]	9	4 (0.97; 0.26–3.68; 0.910)	5 (1.22; 0.36–4.19; 0.950)	2 (1.27; 0.25–6.45; 0.290)	1(1.08; 0.11–10.18; 0.06)
GG Vs AG + AA (dominant model)[a]	73	23 (0.88; 0.48–1.63; 0.690)	26 (1.06; 0.59–1.89; 0.850)	20 (1.68; 0.87–3.22; 0.13)	2 (0.25; 0.05–1.19; 0.046)
AG + GG Vs AA (recessive model)[a]	231	81(1.00; 0.27–3.78; 1.000)	81 (1.21; 0.36–4.13; 0.760)	45 (1.05; 0.21–5.22; 1.00)	21 (1.48; 0.16–13.83; 0.740)
GG + AA Vs AG (over-dominant model)[a]	176	66 (0.87; 0.46–1.66; 0.670)	65 (1.01; 0.54–1.89; 0.970)	29 (1.71; 0.88–3.33; 0.120	21(0.14; 0.02–1.12; 0.018)

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphisms.
[a]OR and *P*-value were adjusted for covariates (age, sex, residential environment and smoking habit of father).

Table 4 Effect of genotype on asthma risk stratified by industry/factory in nearby residence, smoking habit in father and family history of asthma

	Industry/factory				*Smoking habit*				*Family history of asthma in asthmatics*	
	Yes		*No*		*Yes*		*No*			
Genotypes	N, *case/control*	*OR (95% CI); P-value*	N, *case/control*	*OR (95% CI); P-value*	N, *case/control*	*OR (95% CI); P-value*	N, *case/control*	*OR (95% CI); P-value*	*Yes* (N = *103*), *No (137)*	*OR (95% CI); P-value*
rs4794067										
CC	04/5	1	19/59	1	4 /19	1	19/44	1	8/15	1
CT	15/4	3.34 (0.43–25.56); 0.244	70/110	1.95 (1.07–3.54); 0.025	20/23	4.44 (1.26–15.61); 0.240	65/91	1.65 (0.88–3.09); 0.116	39/46	1.48 (0.57–3.80); 0.411
TT	17/3	11.33 (1.41–91.03); 0.022	115/59	5.91 (3.22–10.84); 0.001	26/10	14.35 (3.76–54.80); 0.001	104/52	4.53 (2.40–8.55); 0.001	56/76	0.80 (0.46–1.40); 0.445
rs16947078										
GG	28/9	1	141/158	1	34/34	1	135/132	1	79/90	1
AG	08/2	1.05 (0.15–7.02); 0.956	51/62	0.92 (0.59–1.43); 0.731	14/15	1.00 (0.40–2.44); 0.986	45/49	0.89 (0.55–1.43); 0.639	20/39	0.54 (0.15–1.91); 0.341
AA	00/1	NC	121/8	1.71 (0.68–4.34); 0.253	4/3	1.54 (0.31–7.68); 0.595	8/6	1.31 (0.43–3.92); 0.626	4/8	0.86 (0.22–3.30); 0.830

Abbreviations: CI, confidence interval; OR, odds ratio.
OR and *P*-value were calculated by binary logistic regression and adjusted for covariates age and sex.

Table 5 Distribution of haplotype frequencies in studied subjects and association with risk of asthma

				Male			*Female*		
Haplotypes	*Cases*	*Controls*	*OR (95% CI)*, P-*value*	*Cases*	*Controls*	*OR (95% CI)*, P-*value*	*Cases*	*Controls*	*OR (95% CI)*, P-*value*
CG	0.213	0.417	1	0.209	0.404	1	0.221	0.441	1
TG	0.613	0.411	2.83 (2.10–3.82), 0.001	0.618	0.429	2.78 (1.92–4.02), 0.001	0.603	0.377	3.22 (1.93–5.40), 0.001
TA	0.113	0.084	2.54 (1.58–4.07), 0.001	0.118	0.086	2.67 (1.50–4.74), 0.001	0.102	0.100	2.08 (0.93–4.64), 0.720
CA	0.059	0.086	1.35 (0.78–2.31), 0.273	0.052	0.079	1.26 (0.63–2.54), 0.051	0.072	0.080	1.76 (0.72–4.33), 0.215

Abbreviations: CI, confidence interval; OR, odds ratio.
OR and *P*-value were calculated by binary logistic regression.

and without smoking habit (OR = 14.35, 95% CI 3.76–54.80; $P = 0.001$ and OR = 4.53, 95% CI 2.40–8.55; $P = 0.001$, respectively). On the other hand, subjects with TT genotype, along with smoking habit of father showed 3.2-fold higher risk of asthma in comparison to non-smoker father. Likewise, subjects homozygous and heterozygous of rs4794067 polymorphism, without industry nearby residence also had significantly higher risk (OR = 5.91, 95% CI 3.22–10.84; $P = 0.001$ and OR = 1.95, 95% CI 1.07–3.54; $P = 0.025$) of asthma. While only homozygous (TT) individuals who were nearby resident of industry were found significantly associated with asthma (OR = 11.33; 95% CI 1.41–91.03; $P = 0.022$). However, interaction analysis for the rs16947078 polymorphism did not exhibit any association.

DISCUSSION

Asthma is a multifactorial disease influenced by both environment and genetic factors. Recent studies showed the importance of SNPs of low penetrance genes in predicting risk of asthma. Thus, it is anticipated that identification of SNPs and their association with asthma may help in the development of new therapeutic strategies.[1,2,33–35]

Asthma is the outcome of complex interactions between cytokines, transcription factors and signaling pathways. One such transcription factor TBX21 is considered as a master controller of airway immunopathology in asthma.[5,10] It serves as a regulator of Th1 cell development both by activating interferon-γ production and by reducing Th2 cytokines (IL-4 and IL-5).[7] *TBX21*$^{-/-}$ knockout mice study showed infiltrated bronchi with eosinophils, lymphocytes and airway remodeling even in the absence of allergic sensitization.[13] Study on twins observed a strong genetics influence on expression of TBX21.[14,16] Additionally, the decreased expression of TBX21 was observed in the asthmatics in comparison to controls and demonstrated the potential role of TBX21 in the association of asthma.[5,20]

A recent pharmacogenetic study showed an association between *TBX21* gene variants and inhaled corticosteroid responsiveness as well as lower bronchial hyperresponsiveness in asthmatic children.[33] Other association studies have also confirmed the role of transcription factor *TBX21* gene variants in asthmatics.[18–23] Thus it is conceivable that genetic variation of *TBX21* gene may also affect degree of bronchial hyperresponsiveness, disease severity and therapeutic response in these children. However, results of genetic association studies differ with ethnicity[17] mandating the necessity of such studies from different populations.

To our knowledge, this is the first study describing the association of rs4794067 and rs16947078 polymorphisms in the *TBX21* gene with asthma and its severity in Indian children. We conducted a case–control study in North Indian children aged 1–15 years, all of who were taken from same ethnic group. The present data showed the higher prevalence of asthma in urban area, which may be the result of urban lifestyle and increasing exposure to pollutant.[35,36] We observed that, smoking habit of father significantly influenced asthma risk in children. In accordance with earlier reported study[37–39] we found that use of unprocessed bio-fuel for cooking, residence nearer to vehicular traffic or industry/factory significantly increases the risk of asthma in children. These results suggest the environmental role in asthma progression.

As described in Table 1, there are significant differences observed in living area and smoking by father between cases and controls and these are possible confounders. Therefore to control all possible confounders, adjusted ORs were determined by SNPStats[40] (a web tool for the analysis of association study) including covariates age, sex, living area and smoking habit of father. In the present study we observed a strong association between heterozygous (CT) and homozygous (TT) genotype of rs4794067 polymorphism and asthma.

Children with variant (TT) genotype were at higher risk as compared to those with heterozygous (CT) genotype. Similarly, case–control analysis between genotypes and smoking habits of father/residence near a factory or industry revealed significant association with variant (TT) genotype. However, this genotype was uniformly associated with risk of disease across all the severity groups of asthma. These results provide convincing evidence of the significant involvement of the rs4794067 polymorphism in development of asthma in North Indian children.

It has been suggested that studying haplotypes could be more informative than the study of individual SNPs.[41] In our study, frequency of TG and TA haplotypes was significantly higher in patients and may be associated with the risk of developing asthma. The haplotype interaction analysis with covariate sex also showed significant association of TG and TA haplotypes with asthma in group of males. However, only TA haplotype was significantly associated with asthma in females. In contrast, the rs16947078 polymorphism did not exhibit significant association in our cohort. However, Munthe-Kaas *et al.*[23] reported significant risk between rs16947078 polymorphism and allergic asthma. One possibility is that the effect of this polymorphism varies between ethnic groups.

Although *TBX21* might have played a key role in asthma through airway inflammation, its precise role in asthma susceptibility remains to be explored. Our data strongly support the role of genetic variations in development of asthma in North Indian children and strengthen the hypothesis that genetic polymorphisms of *TBX21* gene control asthma phenotypes.

Our study has several strong points in that, both *TBX21* polymorphisms in our control subjects followed Hardy–Weinberg

equilibrium, all patients were proven cases of asthma, the study subjects enrolled were of similar ethnicity (North Indian) and the possibility of population admixture was ruled out. In addition, stringent quality control and reproducible genotyping measures were used to minimize systematic errors. A limitation of the study is the small number of patients used due to the low incident rate of asthma in our country. The aim of the power calculation was to show the probability of a type 2 error in the recruited sample sizes of the present study. We did not observe any association between asthma and rs16947078 polymorphism, this may be due to low power to detect an association. However, the sample numbers of our study was adequate to achieve sufficient statistical power to detect an association between asthma and rs4794067 polymorphism.

Based on our results, we conclude that *TBX21* gene polymorphism was associated with an increased risk of asthma. Determination of *TBX21* genotype may provide a useful genetic marker in predicting individuals at higher risk for development of asthma. However additional population-based studies with larger sample size are needed before applying these in clinical application.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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