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Molecular Section Original Article

ASSOCIATION OF A DISINTEGRIN AND METALLOPROTEASE 33 GENE POLYMORPHISMS AND THEIR HAPLOTYPES WITH ASTHMA IN THE NORTH-INDIAN POPULATION

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ABSTRACT

AIMS, SETTINGS, AND DESIGN: *A disintegrin and metalloprotease 33 (ADAM33), was the first identified asthma-susceptible gene by positional cloning. A case-control study was performed. We investigated: (1) Association of ADAM33 gene polymorphisms (V2 C/T, T2 A/G, T1 A/G, Q-1 A/G, BC+1 A/G and S1 A/G) with asthma and its severity. (2) The distribution of ADAM33 gene polymorphisms' haplotypes in the studied population and their association with the risk of asthma.* ***SUBJECTS AND METHODS:*** *Using polymerase chain reaction and restriction fragment length polymorphism, six polymorphic sites V2 (C/T), T2 (A/G), T1 (A/G), BC+1 (A/G), Q-1 (A/G), and S1 (A/G) were genotyped in 390 controls and 386 cases of asthma to investigate their association with asthma. Among the recruited cases, 95 (24.6%) were mild intermittent, 235 (60.9%) were mild persistent and 56 (14.5%) were moderate persistent.* ***STATISTICAL ANALYSIS USED:*** *The whole analysis was age- and gender-adjusted. Logistic regression model was used to find out the contribution of genetic polymorphisms to the risk of disease.* ***RESULTS AND CONCLUSION:*** *We found statistically significant association of single nucleotide polymorphisms (SNPs) T1, S1, and T2 with asthma; however, none of the SNPs were found to be associated with the severity of asthma. GTGGGG haplotype was associated with the risk of asthma (Odds ratio = 4.40; $P < 0.0001$). In conclusion, results suggest the importance of ADAM33 SNPs with asthma in the North-Indian population.*

Key words: *A disintegrin and metalloprotease 33, association, asthma, haplotype, single nucleotide polymorphism*

INTRODUCTION

Asthma is the most common chronic disease, affecting nearly 155 million individuals worldwide, both children and adults.[1] Asthma is a complex genetic disorder with several overlapping phenotypes.[2] It is defined as chronic inflammatory disorder of the airways, associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. Variable airflow obstruction that occurs within the lung during an attack is often reversible either spontaneously or with treatment.[3] In India, the prevalence of asthma in adults is 2.38%[4] and in children it differs from 3% to 31%.[5,6]

A disintegrin and metalloprotease 33 (ADAM33) is the first reported asthma-susceptible gene located on chromosome 20p13 identified by positional cloning.[7] ADAM33 consists of 22 exons and is predicted to have protease activity and a domain structure composed of a signal sequence, prodomain, metalloprotease domain (with a zinc-binding motif), disintegrin domain, cysteine-rich domain (with an Epidermal Growth Factor (EGF)-like motif), a transmembrane domain, and a cytoplasmic domain.[8] From a functional standpoint, these different domains interpret into different functions of ADAM33, which include role in cell–cell and cell–matrix interactions,[9] cell migration,[10,11] cell–cell adhesion,[12] and signal transduction.[13] The ADAMs protein family constitutes a variety of cell-surface proteins, including growth factors, cytokines, and receptors.[9]

The original description of ADAM33 as an asthma candidate gene in two Caucasian populations from the UK and the USA,[7] identified a locus on the short arm of chromosome 20 and assessed 135 polymorphisms of 23 genes in this region and reported the ADAM33 gene to be significantly associated with asthma.[7] A number of studies have been published with very diverse results, suggesting its role in asthma.[7,14-31] However, results were not similar in all the studied populations, as some studies showed a positive association of ADAM33 polymorphisms with asthma and another different set of studied populations were not associated with asthma. There is a need to study all of the most common single nucleotide polymorphisms (SNPs) association with asthma together with gene-environment and clinical factors worldwide to find out which are universally associated with asthma. Therefore, the aims of present study were:[1] To assess association of ADAM33 gene polymorphisms (V2 C/T, T2 A/G, T1 A/G, Q-1 A/G, BC+1 A/G and S1 A/G) with asthma and its severity.[2] To assess the distribution of ADAM33 gene polymorphisms' haplotypes and association with risk of asthma.[3] Among

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asthmatics, to assess association with family history of asthma.

SUBJECTS AND METHODS

Study participants

A total of 776 subjects, including 386 asthmatics cases and 390 non-asthmatic healthy controls were enrolled in this study, between August 2007 and September 2009 from the Department of Pediatrics and Department of Pulmonary Medicine, Chattrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India. The study was approved by the institutional ethics committee and a written informed consent for participation was obtained from the subjects/ parents/guardians of all the patients and controls and our study.

Characterization of phenotype

The diagnosis and classification of the asthma severity was made according to the Global Initiative for Asthma guidelines (GINA, 2005).[3] The cases were characterized on the basis of the presence of the following inclusion criteria: (a) Diagnosis of asthma according to the treating physician, (b) symptoms, (c) use of medications for asthma, and (d) reversible airflow limitation shown by spirometry values peak expiratory flow and forced expiratory volume (FEV1 in the 1st s). For <5 years of age, spirometry was not possible, therefore, the diagnosis of asthma was based on the presence of clinical symptoms as given in GINA guidelines, and for stringent criteria of selecting asthma cases <5 years of age, cases were recruited that showed the presence of two or more of the following symptoms: (a) Current presence of wheeze in any child with a history of more than three episodes of documented wheeze or use of bronchodilator in the preceding 12 months or (b) on any regular medication for asthma such as corticosteroids, b-2 agonist, methyl xanthines, leukotriene modifiers, and cromones or (c) currently hospitalized and diagnosed as a case of bronchial asthma and (d) presenting with symptoms of asthma along with a positive family history of asthma.

Controls were selected from the same source population and population was homogeneous with regard to ethnic background. The inclusion criteria for controls were: (a) No past or present physician diagnosis of asthma and other pulmonary disease; (b) no history of wheezing, shortness of breath, and other symptoms of allergic diseases such as nasal and skin symptoms; (c) no use of medications for asthma; and (d) absence of first-degree relatives with a history of asthma. Demographic data were obtained through personal interviews using a standard performa.

Deoxyribonucleic acid extraction and genotyping

Genomic deoxyribonucleic acid (DNA) obtained from 3 ml of venous blood collected in ethylenediaminetetraacetic acid, was extracted using a commercially available kit (FlixiGene DNA Kit, QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol and following the manufacturer's instructions. Six SNPs of the ADAM33 were selected for the screening. These include rs3918400 (V2) C/T, rs2280090 (T2) A/G, rs2280091 (T1) A/G, rs487377 (BC+1) A/G, rs612709 (Q-1) A/G, and rs3918396 (S1) A/G (accessed through Entrez SNP database: http://www.ncbi.nlm.nih.gov/snp/) [Figure 1]. Genotyping was done by polymerase chain reaction–restriction fragment length polymorphisms (PCR–RFLP). Using isolated genomic DNA as a template, PCRs were carried out. Each PCR was performed in a total reaction volume of 15 μl, with 20 pmol of each primer sequence. The PCR products were digested with 1 U of restriction enzymes according to the manufacturer's protocol and following the manufacturer's instructions [Figure 2]. Detailed information on PCR conditions, primer sequences, and restriction enzymes are summarized in Table 1. Twenty-five percentages of samples from patients and controls including samples of each genotype were re-genotyped and no discrimination was found.

Statistical analysis

Data were analyzed using software SPSS 11.5 (SPSS Inc., Chicago, IL) and Epi Info (available from the centers for Disease control and Prevention Atlanta, GA, USA; http://

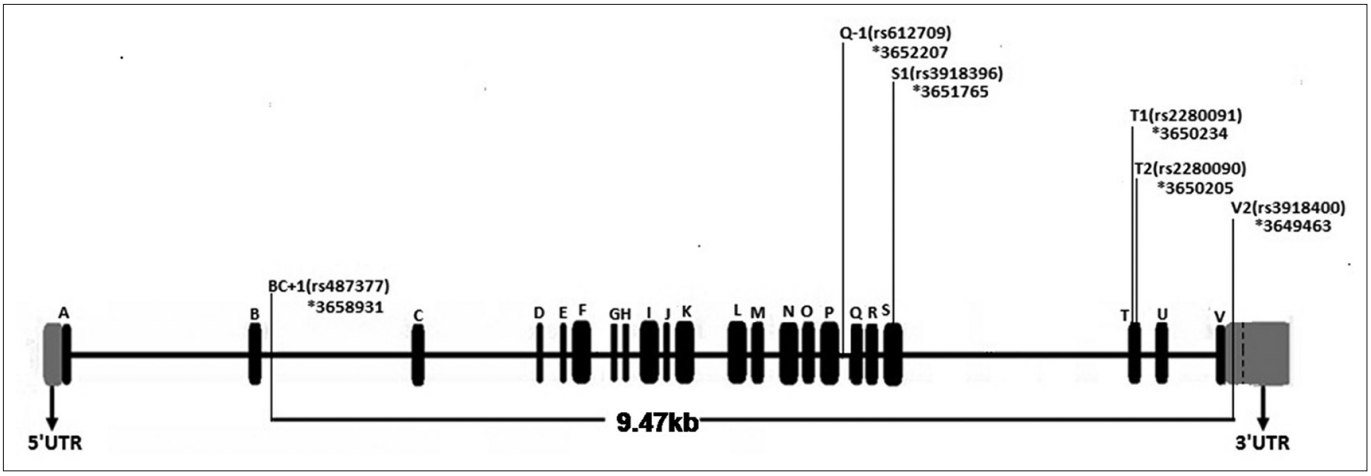

Figure 1: Region covered by six genotyped polymorphisms single nucleotide polymorphisms (SNPs) in kilobases, (a) Position of the genotyped polymorphisms SNPs in the a disintegrin and metalloprotease 33 gene with respect to the 22 exons (black) and untranslated regions (gray) of the gene. *Represents SNPs position on chromosome

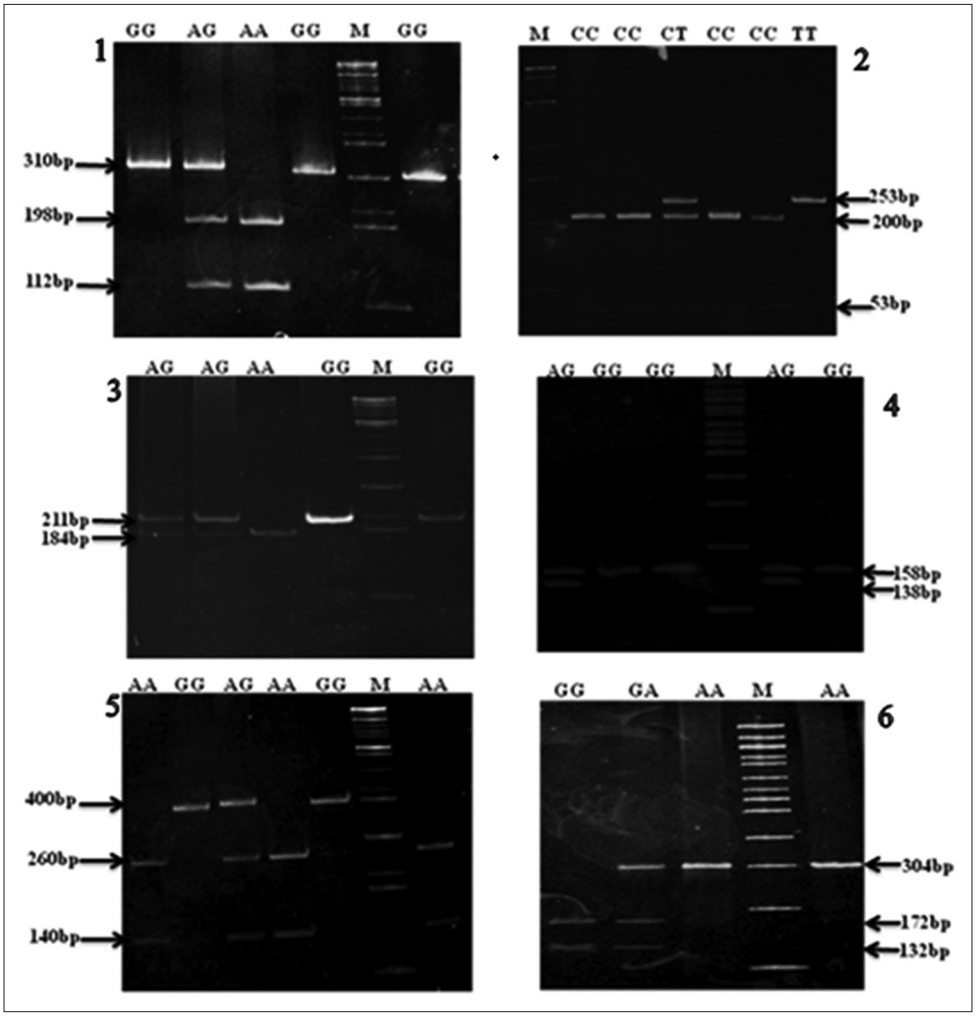

Figure 2: M=100bp marker 1) T2 A/G (Genotypes: AA=Homozygous Normal, AG=Heterozygous, GG=Homozygous Mutant) 2) V2 C/T (Genotypes: CC=Homozygous Normal, CT=Heterozygous, TT=Homozygous Mutant) 3) BC+1 A/G (Genotypes: AA=Homozygous Normal, AG=Heterozygous, GG=Homozygous Mutant) 4) Q-1 A/G (Genotypes: AA=Homozygous Normal, AG=Heterozygous, GG=Homozygous Mutant) 5) T1 A/G (Genotypes: AA=Homozygous Normal, AG=Heterozygous, GG=Homozygous Mutant) 6) S1 A/G (Genotypes: AA=Homozygous Normal, AG=Heterozygous, GG=Homozygous Mutant)

Table 1: Description of the investigated a disintegrin and metalloprotease 33 single nucleotide polymorphism

SNP Rs ID (name[c])	*Forward (F) and reverse (R) primers for PCR*	*PCR program*	*Digest (bp) enzyme*
[a]Rs3918400 (V2) C/T	5′ TCCTCCTCATTCTCAGCAGAT3′ (F) 5′ CCAGCCCTCAGGAACTTCTA3′ (R)	94°C 5 min; 40 cycles; 94°C 30 s; 55°C 30 s; 72°C 30 s; 72°C 5 min	TaqI C allele: 200+53 T allele: 253
[b]Rs2280090 (T2) A/G (AAC=Pro77Ser)	5′-TTCTCAGGGTCTGGGAGAAA3′ (F) 5′-GCCAACCTCCTGGACTCTTA-3′ (R)	94°C 5 min; 40 cycles; 94°C 30 s; 52°C 30 s; 72°C 30 s; 72°C 5 min	HpyCH4III A allele: 198+112 G allele: 310
[b]Rs2280091 (T1) A/G (AAC=Met764Thr)	5′-ACTCAAGGTGACTGGGTGCT-3′ (F) 5′-GAGGGCATGAGGCTCACTTG-3′ (R)	94°C 5 min; 40 cycles; 94°C 30 s; 54°C 30 s; 72°C 30 s; 72°C 5 min	NcoI A allele: 140+260 G allele: 400
[a]Rs487377 (BC+1) A/G	5′-GTACAGAAGAAAGAGTAGAGGCTAGAT-3′ (F) 5′GCTCATGAGGCTGGAATGCCACT-3′ (R)	94°C5 min; 40 cycles; 94°C 30 s; 8.4°C 30 s; 72°C 30 s; 72°C 5 min	EcoRV A allele: 184+27 G allele: 211
[b]Rs612709 (Q-1) A/G	5′-GGATTCAAACGGCAAGGAG-3′ (F) 5′-GTTCACCTAGATGGCCAGGA-3′ (R)	94°C 5 min; 40 cycles; 94°C 30 s; 60°C 30 s; 72°C 30 s; 72°C 5 min	HinfI A allele: 20+138 G allele: 158
[b]Rs3918396 (S1) A/G	5′-TGTGCAGGCTGAAAGTATGC-3′ (F) 5′-AGAGCTCTGAGGAGGGGAAC-3′ (R)	94°C 5 min; 40 cycles; 94°C 30 s; 50°C 30 s; 72°C 30 s; 72°C 5 min	BtsCI G Allele: 132+172 A allele: 304

PCR-RFLP=Polymerase chain reaction-restriction fragment length polymorphisms, a=The PCR-RFLP method to detect the SNP was developed in the present study, b=The PCR-RFLP method to detect the SNP was taken from study, Su *et. al.*, 2008,[15] c=SNP names refer to those used by Van Eerdewegh *et al.*, AAC=Amino acid change, SNPs=Single nucleotide polymorphism, ID=Identification details polymerase chain reaction

www.cdc.gov/epo/epi/epiinfo.htm). The whole analysis was age- and gender-adjusted. $P < 0.05$ was considered significant. Demographic characteristics of patients and controls were described as frequencies and percentages, whereas descriptive statistics of patients and controls were presented as mean and standard deviations for continuous measures. Data were compared by Chi square (χ^2) test and Fisher's exact test. Logistic regression models were used to calculate the odds ratio (OR).[32] Any deviation from Hardy–Weinberg equilibrium was calculated by a Chi square (χ^2) goodness-of-fit test. *P* values were corrected (Pcorr) for multiple corrections (Bonferroni correction).[32,33] Haplotype

frequencies and linkage disequilibrium were calculated by expectation–maximization algorithm[34] using Arlequin software version 2.000 (University of Berne, Switzerland) and SNP Analyzer Version 1.0 (ISTECH; Istech, Kyungkido, Republic of Korea).

RESULTS

A total of 386 asthmatic patients were grouped into the following categories: (i) Mild intermittent ($n = 95$), (ii) mild persistent ($n = 253$), (ii) moderate persistent ($n = 56$) and (iii) severe persistent (no such cases). A total of 390 non-asthmatic controls were selected from the same source population. Table 2 illustrates the demographics of the study population.

Association of a disintegrin and metalloprotease 33 gene polymorphisms with asthma

The genotype and allele frequencies of the ADAM33 gene polymorphisms are shown in Table 3. To rule out any possible sampling bias, the Hardy–Weinberg equilibrium (HWE) was calculated separately for both cases and control groups. All the six SNPs that were genotyped were in HWE ($P > 0.05$). Bonferroni's corrections were applied to account for multiple testing issues.

After comparing the genotype and allele frequencies between patients and controls, increased risk for asthma was observed with the heterozygous genotype (AG), homozygous mutant genotype (GG), and mutant allele (G) of SNPs T2 (GA, AA, and A respectively), T1 (GA, AA, and A respectively), and S1 (GA, AA, and A respectively). SNPs V2, Q-1, and BC+1 were not found to be associated with asthma. Nevertheless, the mutant allele (G) of SNP BC+1 showed a protective association with asthma (Patients (G):79.3%; Controls (G):84.0), (OR = 0.7; 95% CI = 0.6-0.9; $P = 0.017$).

A "case only" study was performed to test a possible genotypic distribution pattern in patients with a positive family history of asthma and those who are without a family history of asthma and found none to be associated. Likewise, an analysis performed to assess the association of genotypes namely; homozygous normal, heterozygous, and homozygous mutant with hospitalization of asthma; however, did not find any association. (Insignificant data not shown.)

Association of a disintegrin and metalloprotease 33 polymorphisms with severity of asthma

The genotype frequencies of the six studied polymorphisms of ADAM33 with severity categories of asthma are shown in Table 4. To find out association with the severity of asthma, we did a separate analysis for each of the high severity groups, namely, mild persistent and moderate persistent with the low severity group named as mild intermittent and observed none of the SNPs to be statistically significantly associated with asthma. However, on analyzing the V2 SNP, we observed that the OR of mutant genotype was related to the severity of asthma (Mild Intermittent vs. Mild Persistent [OR = 1.4; $P = 0.305$] and Mild Intermittent vs. Moderate Persistent [OR = 2.4; $P = 0.091$]). The association was most significant in the moderate persistent category as compared to its associations with mild persistent. This finding indicates that the association was related to the increasing severity of asthma.

Association of a disintegrin and metalloprotease 33 haplotypes with risk of asthma

All the 386 cases and 390 controls were included for the haplotype analysis. After the estimation of haplotypes, a total of 42 haplotypes were found in asthmatic patients and in controls. Certain haplotypes, although present in the healthy controls, were altogether absent in patients or vice versa. Thus, those patients and healthy controls could not be included for the haplotype analysis. In addition, for statistical advantage, haplotypes with a frequency of <1% were bundled together in a group of "rare." The frequencies of haplotypes between cases and controls are shown in Table 5. The GTGGGG (haplotype 1) haplotype was found to be associated with asthma (OR = 4.40; 95% CI = 2.93-6.65 [$P < 0.0001$]). Haplotypes that were protective against asthma were; GTAGGG (haplotype 5), (OR = 0.7; 95% CI = 0.4-1.0) GTGAAG (haplotype 9) ($P = 0.048$, OR = 0.6; 95% CI = 0.4-0.9), GTGAGG (haplotype 11) ($P = 0.026$, OR = 0.4; 95% CI = 0.3-0.7; $P = 0.001$), and GTAAAG (haplotype 15) (OR = 0.2; 95% CI = 0.1-0.5; $P < 0.0001$). The remaining haplotypes had comparable frequency among patients and healthy controls. Two sets of controls; V2/T1 and S1/T2 were in LD. (Yates's corrected $P < 0.05$ and $|D'| \neq 0$).

Table 2: Demographic profile of selected population

Basic demographic	*(N=776)*				
	Controls (390)	*Cases (386)*	*Severity subgroups of asthma (N)*		
			Mild intermittent (95)	*Mild persistent (235)*	*Moderate persistent (56)*
Gender (female) *N* (%)	93 (23.8)	122 (31.6)	28 (29.5)	73 (31.1)	21 (37.5)
Age (in years) M±SD	22.87±14.54	18.7±15.9	18.32±13.87	18.16±15.76	21.28±19.47
Weight (in Kg) M±SD	46.75±22.94	36.5±22.4	37.18±21.14	36.36±22.42	35.88±24.62
Height (in cm) M±SD	145.5±28.9	133.3±30.8	136.39±29.29	132.37±31.97	131.93±28.46
BMI M±SD	20.0±5.4	18.3±6.6	17.93±4.69	18.52±7.34	18.05±6.51
Hospitalization for asthma *N* (%)		157 (40.7)	14 (14.7)	102 (43.4)	41 (73.2)
Family history of asthma *N* (%)		174 (45.1)	44 (46.3)	102 (43.4)	28 (50.0)

N (%)=Number (percentage), M±SD=Mean±standard deviation, BMI=Body mass index

Table 3: Association of the studied polymorphisms of a disintegrin and metalloprotease 33 gene with asthma in the Indian population

SNPs	*Genotypes*	*Patients (386)*		*Controls (390)*		*OR (95% CI) P value*
		(N)	*(%)*	*N*	*(%)*	
Q-1 A>G	Genotypes					
	AA	0	0	0	0	-
	AG	60	15.5	60	15.4	Reference[1]
	GG	326	84.5	330	84.6	1.0 (0.7-1.5) 0.926
	Alleles					
	A	60	7.8	60	7.7	Reference[1]
	G	712	92.2	720	92.3	0.9 (0.7-1.4) 0.953
V2 C>T	Genotypes					
	CC	69	17.9	60	15.4	Reference[1]
	CT	167	43.3	197	50.5	0.8 (0.6-1.3) 0.385
	TT	150	38.9	133	34.1	1.2 (0.8-1.8) 0.505
	Alleles					
	C	305	39.5	337	43.2	Reference[1]
	T	467	60.5	443	56.8	1.2 (0.9-1.4) 0.139
T1 A>G	Genotypes					
	AA	58	15.0	100	25.6	Reference[1]
	AG	170	44.0	183	46.9	1.6 (1.1-2.4) 0.013
	GG	158	40.9	107	27.4	2.8 (1.8-4.2) <0.001
	Alleles					
	A	286	37.0	383	49.1	Reference[1]
	G	486	63.0	397	50.9	1.6 (1.3-2.0) <0.001
S1 A>G	Genotypes					
	AA	44	11.4	95	24.4	Reference[1]
	AG	163	42.2	177	45.4	2.1 (1.4-3.3) <0.001
	GG	179	46.4	118	30.3	3.6 (2.4-5.6) <0.001
	Alleles					
	A	381	49.4	367	47.1	Reference[1]
	G	391	50.6	413	52.9	1.8 (1.5-2.3) <0.001
T2 A>G	Genotypes					
	AA	54	14.0	101	25.9	Reference[1]
	AG	168	43.5	192	49.2	1.6 (1.1-2.4) 0.020
	GG	164	42.5	97	24.9	3.2 (2.1-4.8) <0.001
	Alleles					
	A	276	35.8	384	49.2	Reference[1]
	G	496	64.2	396	50.8	1.8 (1.5-2.2) <0.001
BC+1 A>G	Genotypes					
	AA	12	3.1	6	1.5	Reference[1]
	AG	136	35.2	113	29.0	0.6 (0.2-1.6) 0.260
	GG	238	61.7	271	69.5	0.4 (0.1-1.0) 0.062
	Alleles					
	A	160	20.7	125	16.0	Reference[1]
	G	612	79.3	655	84.0	0.7 (0.6-0.9) 0.017

N=Number, %=Percentage, OR=Odds ratio, SNPs=Single nucleotide polymorphisms

DISCUSSION

In this study, we analyzed six polymorphisms (T1, T2, and V2, S1, BC+1, and Q-1) and their haplotypes in ADAM33 to investigate their association with asthma.

We have found the association of ADAM33 SNPs T2, T1, and S1 with asthma. However, none of the studied SNPs showed statistically significant association with the severity of asthma. We also observed a haplotype containing a mutant allele of all studied SNPs GTGGGG, with asthma.

The results of the comparisons of studied SNPs (T1, T2, and V2, S1, BC+1, and Q-1) with other studied populations are summarized in Table 6. Van Eerdewegh *et al*., in the year 2002,[7] identified a locus on the short arm of chromosome 20 and assessed 135 polymorphisms of 23 genes in this region and reported the ADAM33 gene to be significantly associated with asthma,[7] similarly, we observed significant association with SNPs T1, T2, and S1with asthma. In another case-control study, Howard *et al*.[27] assessed the association of eight SNPs of ADAM33 including SNPs S1, T1, and T2 with asthma in four unique ethnic groups viz.: U.S. white, Dutch white, African-American, and Hispanic. They observed associations between presence of asthma among the U.S. white people with SNPs T1 ($P = 0.03$); T2 ($P = 0.02$) and in U.S. Hispanics with SNP T2 ($P = 0.04$). In contrast to our results, Lind *et al*.[28] studied six SNPs, including S1, T1, and T2 of ADAM33 using the transmission disequilibrium test to analyze associations between the ADAM33 gene variants and asthma, asthma severity, bronchodilator responsiveness, and total Immunoglobulin E (IgE) levels using single SNPs, two to six SNPs combinations, specific haplotypes, but were unable to demonstrate association of any SNPs either with asthma or other outcomes. Another study by Raby *et al*.[25] performed on 17 SNPs of ADAM33, including T1 and T2, was unable to detect an association in a family-based study of White, African-American, and Hispanic trios representative of North-American children with mild-to-moderate asthma. Werner *et al*.[26] analyzed 15 SNPs, of ADAM33 including S1, T1, and T2 and observed that these three were not statistically associated with asthma. Blakey *et al*.[23] conducted transmission disequilibrium and case-control studies in Icelandic and Nottingham study populations and but did not find any association with SNPs included in present study. Similarly, a study conducted on the Chinese population by Wang *et al*.[20] did not find any association of SNP T1 with asthma. Another study by Schedel *et al*.[21] analyzed ten SNPs, including S1, T1, and T2, however found none to be significantly associated with asthma in both case-control and cohort study designs in the German population.

Similar to our results, a study on Chinese Han population analyzed six SNPs of ADAM33, including our SNPs of interest namely T2, T1, S1, and Q-1, and observed that, T2, T1, and Q-1 increase the risk of susceptibility. The findings of the present study also resemble the above-mentioned findings except Q-1 which was not found to be associated

Table 4: Association of the studied polymorphisms of a disintegrin and metalloprotease 33 gene with severity subgroups of asthma

Genotype	*Mild intermittent N=95 (%)*	*Mild persistent N=235 (%)*	*Mild intermittent versus mild persistent OR (95% CI)*	*Moderate persistent N=56 (%)*	*Mild intermittent versus moderate persistent OR (95% CI)*
Q-1					
AA	0	0	0	0	0
AG	16 (16.8)	35 (14.9)	Reference[1]	9 (16.1)	Reference[1]
GG	79 (83.2)	200 (85.1)	1.2 (0.6-2.2) 0.658	47 (83.9)	1.1 (0.4-2.6) 0.902
V2					
CC	21 (22.1)	41 (17.4)	Reference[1]	7 (12.5)	Reference[1]
CT	41 (43.2)	103 (43.8)	1.3 (0.7-2.4) 0.439	23 (41.1)	1.7 (0.6-4.6) 0.306
TT	33 (34.7)	91 (38.7)	1.4 (0.7-2.7) 0.305	26 (46.4)	2.4 (0.9-6.4) 0.091
T1					
AA	9 (9.5)	43 (18.3)	Reference[1]	6 (10.7)	Reference[1]
AG	48 (50.5)	99 (42.1)	0.4 (0.2-0.9) 0.039	23 (41.1)	0.7 (0.2-2.2) 0.572
GG	38 (40.0)	93 (39.6)	0.5 (0.2-1.2) 0.106	27 (48.2)	1.1 (0.3-3.3) 0.913
S1					
AA	13 (13.7)	28 (11.9)	Reference[1]	3 (5.4)	Reference[1]
AG	39 (41.1)	102 (43.4)	1.2 (0.6-2.6) 0.614	22 (39.3)	2.4 (0.6-9.5) 0.198
GG	43 (45.3)	105 (44.7)	1.1 (0.5-2.4) 0.742	31 (55.4)	3.1 (0.8-11.9) 0.095
T2					
AA	14 (14.7)	31 (13.2)	Reference[1]	9 (16.1)	Reference[1]
AG	44 (46.3)	103 (43.8)	1.1 (0.5-2.2) 0.880	21 (37.5)	0.7 (0.3-1.9) 0.554
GG	37 (38.9)	101 (43.0)	1.2 (0.6-2.6) 0.577	26 (46.4)	1.1 (0.4-2.9) 0.858
BC+1					
AA	4 (4.2)	7 (3.0)	Reference[1]	1 (1.8)	Reference[1]
AG	40 (42.1)	77 (32.8)	1.1 (0.3-3.9) 0.885	19 (33.9)	1.9 (0.2-18.2) 0.577
GG	51 (53.7)	151 (64.3)	1.7 (0.5-6.0) 0.417	36 (64.3)	2.8 (0.3-26.3) 0.362

N=Number, %=Percentage, OR=Odds ratio

Table 5: Distribution of genotyped a disintegrin and metalloprotease 33 gene polymorphism's haplotypes and its association with risk of asthma

Total no.	*Haplotype*						*Case (772)[a]*		*Control (780)[a]*		*OR (CI 95%)*
Selected SNPs	*Q-1*	*V2*	*T1*	*S1*	*T2*	*BC+1*	*No*	*%*	*No*	*%*	*P value*
1	G	T	G	G	G	G	129	16.77	34	4.33	4.40 (2.93-6.65) <0.0001
2	G	T	G	G	A	G	63	8.21	76	9.72	0.82 (0.57-1.18) 0.275
3	G	C	G	G	G	G	54	7.02	45	5.74	1.25 (0.80-1.89) 0.323
4	G	C	A	G	A	G	45	5.88	33	4.2	1.40 (0.86-2.28) 0.149
5	G	T	A	G	G	G	37	4.74	56	7.24	0.65 (0.42-1.02) 0.048
6	G	T	A	A	G	G	36	4.7	30	3.84	1.22 (0.73-2.06) 0.425
7	G	C	G	A	A	G	31	4.06	39	4.95	0.79 (0.48-1.32) 0.350
8	G	C	A	A	G	G	29	3.77	34	4.41	0.86 (0.50-1.46) 0.548
9	G	T	G	A	A	G	27	3.53	46	5.93	0.56 (0.35-0.96) 0.026
10	G	T	G	G	G	A	25	3.29	21	2.67	1.21 (0.65-2.27) 0.526
11	G	T	G	A	G	G	24	3.17	56	7.19	0.41 (0.25-0.69) 0.001
12	G	C	A	G	G	G	23	3.01	38	4.81	0.60 (0.34-1.05) 0.058
13	G	C	G	A	G	G	15	1.92	14	1.79	1.08 (0.49-2.39) 0.829
14	G	C	A	G	G	A	12	1.6	10	1.31	1.22 (0.49-3.05) 0.649
15	G	T	A	A	A	G	9	1.19	38	4.81	0.23 (0.10-0.50) <0.0001
16	Rare						209	27.07	212	27.17	0.99 (0.79-1.25) 0.962

[a]=Total no of chromosome RARE haplotypes (frequency >1% in either cases or controls) were bundled together in a group of RARE, SNPs=Single nucleotide polymorphisms, OR=Odds ratio

with asthma in our population.[15] Similar to the results of our study, another study by Kedda *et al*.[19] on 10 SNPs, which also covered SNPs V2 and Q-1 was unable to find a single SNP association with asthma.

Thongngarm *et al*.[29] conducted a study in a Thai population, studied eight SNPs including SNPs S1 and T1, but was unable to find the association of SNPs S1 and T1 with the risk of asthma. Same contradictory results

Table 6: Association of studied a disintegrin and metalloprotease 33 polymorphisms from various populations

Reference	*Study population*	*N[(a)] case/control families (individuals)*	*ADAM33 SNPs and reported association*					
			T1	*T2*	*S1*	*Q-1*	*BC+1[(b)]*	*V2*
Jie *et al*., 2011[30]	East Chinese Han	150/74	+	+	–	*	*	*
Qu *et al*., 2011[31]	Northern Chinese	412/397	+	–	–	–	*	*
Bijanzadeh *et al*., 2010[14]	South India (Mysore) (cc)	100/50	–	*	*	*	*	*
Vergara *et al*., 2010[16]	Cartagena, Colombia (cc)	429/401	–	–	*	*	*	*
	Cartagena, Colombia (fa)	116 (348)	–	–	*	*	*	*
Su *et al*., 2008[15]	Chinese Han (cc)	181/151	+	+	–	+	*	*
Sakagami *et al*., 2007[17]	Japanese (cc)	101/120 (AIA)	–	–	*	*	*	*
		282/120 (ATA)	–	–	*	*	*	*
Noguchi e*t al*., 2006	Japanese (fa)	155 (538)	–	+	–	–	*	–
Kedda *et al*., 2006[19]	Australian (cc)	612/473	*	*	–	–	*	–
Wang *et al*., 2006[20]	Chinese (cc)	296/270	–	*	*	*	*	*
Schedel *et al*., 2006[21]	German (cc)	624/1248	–	–	–	*	*	*
	Cohort (coh)	86/464	–	–	–	*	*	*
Hirota *et al*., 2006[22]	Japanese (cc)	504/651	+	+	*	*	*	*
Blakey *et al*., 2005[23]	Icelandic (cc)	348/262	–	–	–	–	*	*
	Nottingham (fa)	60 (240)	–	–	–	–	*	*
Lee *et al*., 2004[24]	Korean (cc)	326/121	–	*	–	*	*	*
Raby *et al*., 2004[25]	Non-Hispanic white (fa)	474 (1462)	–	–	*	*	*	*
	Hispanic (fa)	47 (149)	+	–	*	*	*	*
	African American (fa)	66 (203)	–	–	*	*	*	*
Werner *et al*., 2004[26]	German (cc)	48/499	–	–	–	–	*	*
	German (fa)	171 (732)	–	–	–	–	*	*
Howard *et al*., 2003[27]	African American (cc)	161/265	–	–	–	*	*	*
	US White (cc)	220/229	+	+	–	*	*	*
	US Hispanic (cc)	113/127	+	+	–	*	*	*
	Dutch White (cc)	180/133	–	–	–	*	*	*
Lind *et al*., 2003[28]	Mexican (cc)	190/160	–	–	–	*	*	*
	Puerto Rican (cc)	183/165	–	–	–	*	*	*
	Mexican/P. Rican (fa)	583 (1749)[3]	–	–	–	*	*	*
Van Eerdewegh *et al*., 2002[7]	US/UK combined (cc)	130/217	*	*	+	+	*	*
	UK (cc)	(not reported)	*	*	+	+	*	*
	US (cc)	(not reported)	+	+	*	*	*	*
	US/UK (fa)	460 (1840)	*	*	+	*	*	*
Present study	India (cc)	386/390	+	+	+	–	–	–

AIA=Aspirin-intolerant asthma, ATA=Aspirin-tolerant asthma, cc=Case control, coh=Cohort study, fa=Family study, +=Association with asthma, –=SNPs which are not significantly associated, *=Not reported, (a)=In case-control studies number of cases and controls, in family studies number of families and individuals, (b)=None of the association study represents BC+1 association with asthma, however, Simpson *et al*.,[36] working on impaired early-life lung function, have shown evidence of a significant causal location between BC+1 and F+1 SNPs, at the 5' end of the gene, Del Mastro *et al*., 2007,[35] while studying the mechanistic role of a disease-associated genetic variant within the ADAM33 asthma susceptibility gene suggested that SNP BC+1 may have an important role in the modulation of ADAM33 gene, ADAM33=A disintegrin and metalloprotease 33, SNP=Single nucleotide polymorphism

were observed for SNPs T1 and T2 from another study done by Vergara *et al*.,[16] on the population of Cartagena, Colombia, and in this study six SNPs were analyzed including T1 and T2. A study on the South-Indian population by Bijanzadeh *et al*.,[14] in children as well as on adults, also failed to find an association between asthma and the T1 SNP of ADAM33. Although a recent study on the East Chinese Han population observed positive association of T2 and T1 with asthma and another study on the Northern Chinese population showed an association of T1 SNP with asthma.[30,31]

A study, identifying SNPs predictive of phenotype using random forests, showed that the highest association of SNP BC+1 with asthma ($P = 0.0027$) was among the Caucasians.[35] As many research articles indicated that asthma commonly originates in early life in association with impaired lung function, it becomes very important to highlight that using linkage disequilibrium mapping, Simpson *et al*.[36] have also found evidence of a significant causal location between BC+1 and F+1 SNPs, at the 5′ end of the gene.

Apparently, associations of various polymorphisms of the ADAM33 gene with asthma and related phenotypes have been found in the different populations that have been studied. In contrast, no single SNP of the ADAM33 gene has been found to be associated with asthma across all the studies conducted so far. It may because of the heterogeneity of disease, as a single disorder may occur due to different mutations within the gene and with a combined effect of gene–gene and gene-environment factors. Consequently, the role of ADAM33 gene polymorphisms, if any, resulting in asthma or its related phenotypes, either alone or as a result of gene–gene or gene-environment interactions is not clear and functional studies are needed in this area.

In conclusion, our data suggest that ADAM33 gene polymorphisms serve as genetic risk factors for asthma in the North-Indian population. However, further studies are needed to confirm the mechanistic and pathophysiological role of ADAM33 SNPs in asthma susceptibility.

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