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ORIGINAL ARTICLE

Genetic variants of ADAM33 are associated with asthma susceptibility in the Punjabi population of Pakistan

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Abstract

Objective: A disintegrin and metalloproteinase 33 (ADAM33) gene has been considered as an asthma susceptibility gene due to its possible role in airway remodeling, abnormal cell proliferation, and differentiation. Association of this gene with asthma has been reported in several genetic studies on various populations. The current study aims to evaluate the association of ADAM33 gene polymorphisms with the risk of asthma in the Punjabi population of Pakistan. **Method:** A total of 101 asthma patients and 102 age-matched healthy controls from Lahore, a city in Punjab, were recruited. ADAM33 single nucleotide polymorphisms (SNPs) T + 1[rs2280089], T2[rs2280090], T1[rs2280091], ST + 5[rs597980], ST + 4[rs44707], S2[rs528557], Q – 1[rs612709], and F + 1[rs511898] were genotyped in both patients and controls using single base extension and capillary electrophoresis-based genetic analyzer. The basic allelic and genotypic model was analyzed for association of the SNPs with asthma using SHEsis software. Haploview software was used to calculate pairwise linkage disequilibrium (LD) among six of the genotyped SNPs. **Results:** Of the 8 SNPs genotyped, only S2[rs528557] showed significant association with asthma (Allele $p=0.0189$, Genotype $p=0.021$). SNPs T + 1[rs2280089], T2[rs2280090], T1[rs2280091], ST + 4[rs44707], S2[rs528557], and Q – 1[rs612709] were found to be in moderate to strong LD. The significantly higher frequency of haplotype “AAGTCG” in healthy controls suggests a protective effect against asthma risk in the studied population ($p=0.0059$). **Conclusion:** These findings suggest that genetic variants of ADAM33 gene may play important roles in asthma susceptibility in the Punjabi population of Pakistan.

Keywords

ADAM33, single nucleotide polymorphisms, asthma, linkage disequilibrium, haplotype, mini sequencing, Punjabi, Pakistan

History

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Introduction

Asthma is a common chronic respiratory disease in which chronic inflammation results in reversible structural changes in airway remodeling and leads to airflow obstruction and bronchial hyper-responsiveness [1]. It is one of the major chronic diseases; globally 1 out of every 250 deaths is due to asthma complications [2]. More than 300 million people suffer from asthma worldwide and it is estimated that this figure will rise to 400 million by 2025 [2–4]. Although the prevalence of asthma varies in different countries, due to its increasing trend this disease has become a major health concern globally [5]. The increasing prevalence of asthma may be attributed to changes in lifestyle, indoor and outdoor

environment, and climate [6]. In Pakistan, the prevalence of asthma is 6% in school going children in south Punjab [7], 31.58% in Islamabad (Punjab), and 15.8% in Karachi (Sindh) [4]. Over all asthma prevalence in Pakistani youngsters is 6–9% in mixed urban populations [8].

Various risk factors have been investigated to try to understand the cause of asthma, but the exact cause remains unknown [9]. Most studies support the hypothesis that both environmental and genetic factors are important etiological factors [10]. It has been reported that asthmatic effects caused by environmental factors may be modulated by single nucleotide polymorphisms (SNPs) or, conversely, that environmental factors may modulate genomic expression by affecting epigenetic modifications, without altering the genomic sequence, thereby altering the asthma phenotype [11]. Twin studies have predicted higher prevalence of asthma in monozygotic as compared to dizygotic twins and have suggested that genetics play a major role in the manifestation of asthma. However, unlike other syndromes, a single gene or set of genes associated with asthma cannot be easily identified. Rather, small contributions of multiple genes,

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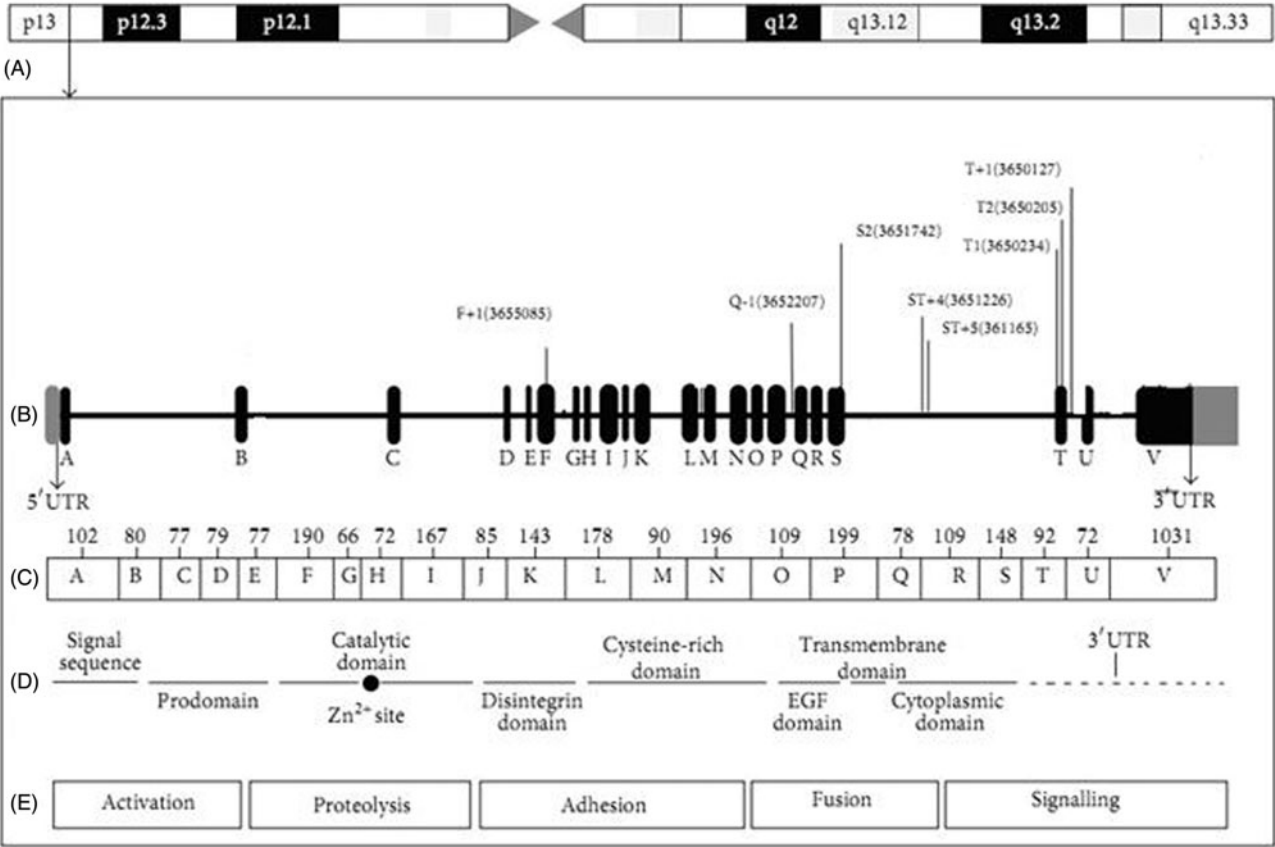


Figure 1. Schematic diagram of the ADAM33 gene on chromosome 20p13 modified from Tripathi et al. [20]. (A) ADAM33 gene location on chromosome 20. (B) Selected SNP positions on ADAM33 gene. (C) ADAM33 exons with sizes in base pairs. (D) Domains of ADAM33 gene. (E) Functional description of ADAM33 domains.

along with environmental factors, have been reported to be involved in asthma susceptibility [12].

A disintegrin and metalloproteinase 33 (ADAM33) gene is known to be involved in the development of asthma and bronchial hyper-responsiveness. ADAM33 was first identified through positional cloning. It is located on chromosome 20p13 and has 22 exons encoding a signal sequence, pro-domain, proteinase catalytic domain, disintegrin domain, cysteine-rich domain, epidermal growth factor-like domain, transmembrane domain, cytoplasmic domain, and a long 3' untranslated region (Figure 1). Gene expression studies have revealed that the removal of ADAM33 function by means of anti-sense oligonucleotides prevents pro-fibrogenic growth factor (TGF- β)-induced differentiation of airway fibroblasts into a contractile phenotype [myofibroblasts] *in vitro* [13–15]. In addition, environmental factors such as passive smoking exposure may interact with ADAM33 in exerting its remodeling function in lungs [16].

Various studies [17] have suggested that more than 100 variants in different target genes, including ADAM33, may be involved in airway remodeling and hyper-responsiveness in diverse populations [18]. Association of various ADAM33 SNPs with risk of asthma has been reported in several ethnic populations, including Indian [19–22], Japanese [23–26], Thai [27], Korean [28], Chinese [29–32], Saudi Arabian [18], Caucasian [33], German [34,35], European [36], and American [37]. The frequent observation of positive association between ADAM33 SNPs and asthma suggests that ADAM33 may be involved in asthma development through

genetic variants [24]. However, conflicting data on association of different SNP variants of ADAM33 with asthma have been reported in different groups of the same populations [30,32,36,38,39]. This variable impact of genetic variants of ADAM33 on risk of asthma in different populations has motivated us to investigate the possible association of ADAM33 SNPs with asthma in the Punjabi population of Pakistan.

In this study, we genotyped the ADAM33 SNPs T + 1[rs2280089], T2[rs2280090], T1[rs2280091], ST + 5[rs597980], ST + 4[rs44707], S2[rs528557], Q – 1[rs612709], and F + 1[rs511898] in asthma patients and healthy controls, using single base extension (SBE) and capillary electrophoresis-based genetic analyzer, to assess their association with risk of asthma in a study population from the Punjabi region of Pakistan.

Materials and methods

Study subjects

A total of 101 asthma patients, diagnosed and screened by physicians, and 102 age-matched healthy controls [age range 4–60 and 6–55 years, respectively] were enrolled in this study from the Asthma and Allergy Clinic, Gulab Devi Chest Institute, Lahore, and The Children's Hospital and The Institute of Child Health, Lahore, after approval by the Technical Evaluation Committee [review board] of the Centre for Applied Molecular Biology, Lahore. Healthy subjects without history of asthma, atopy, and any allergy or any skin

disease were selected to serve as control subjects for the study after screening at the asthma screening unit of The Children’s Hospital, Lahore. Patients and controls with a history of smoking or any other illness involving the lungs, e.g. chronic bronchitis, emphysema, tuberculosis, pneumonia, or any cardiac problem etc. were excluded from this study. Demographic characteristics of the study participants are shown in Table 1.

The study was approved by the Technical Evaluation Committee [review board] of the Centre for Applied Molecular Biology, University of the Punjab, Lahore. Written informed consent was obtained from all subjects (or guardians in case of minors/children) recruited in this study.

Selection of SNP markers

Eight ADAM33 SNPs, T + 1[rs2280089], T2[rs2280090], T1[rs2280091], ST + 5[rs597980], ST + 4[rs44707], S2[rs528557], Q – 1[rs612709], and F + 1[rs511898], were selected for study because they have been reported to be asthma susceptibility variants in various Asian populations [19,31,36,38,39].

DNA extraction and genotyping

Two to four milliliters of peripheral blood samples were collected from patients and controls according to the standard operating procedure. Genomic DNA was extracted from peripheral blood samples using a standard phenol, chloroform, and isoamyl alcohol protocol. Extracted DNA was quantified on agarose gels and by spectrophotometry using a SpectraMax384 (Molecular Devices, Sunnyvale, CA). The

genomic regions containing the selected SNPs were amplified in a multiplex (8plex) PCR. The primer sequences used for multiplex PCR and the sizes of the PCR products are shown in Table 2. PCR reactions were performed in a total volume of 10.0 µL containing 12.5 ng DNA, 0.5 µL of 4 mM dNTPs (Fermentas) , 0.6 µL of 25 mM MgCl₂ (Fermentas, Ontario, Canada), 2.0 µL of primer mix (Biobasic, Ontario, Canada) (4–8 mM each), and 1 µL of 10X PCR buffer (Fermentas) with 1.0U of Taq DNA Polymerase (Fermentas). Samples were denatured at 95 °C for 5 min followed by 30 cycles of 95 °C for 45 s, 58 °C for 45 s, 72 °C for 90 s, and a final extension at 72 °C for 10 min.

PCR products were further processed for SNP genotyping using ABI Prism SNaPshot™ SBE multiplex kit (Applied Biosystems, Foster City, CA). This kit contains ddNTPs labeled with four different fluorescent dyes (i.e. blue, green, red, and yellow/black). A list of the primers used for SNaPshot PCR is given in Table 3. Poly GACT tails of different lengths were added to the 5’ end of the primers to differentiate their PCR products by size. 5 µL of multiplex PCR products from the above reactions were incubated with 1.6U of Exonuclease I (Fermentas) and 2.56U of shrimp alkaline phosphatase (Fermentas) at 37 °C for an hour followed by heating at 80 °C for 15 min to inactivate both enzymes. Following this, the reaction mix containing 2.5 µL of SNaPshot SBE multiplex kit, 3.0 µL of Exo SAP treated PCR products, 1 µL of nuclease free water, and 2.0 µL of primers mix (4–8 mM) was incubated for 25 cycles at 96 °C for 10 s, 56 °C for 5 s, and 60 °C for 30 s. Finally 0.6U of SAP (Fermentas) was added to the reaction product and incubated at 37 °C for an hour to remove the unused primers and unwanted PCR reagents. SAP was then denatured by heating at 75 °C for 15 min. The final reaction mix containing 7.9 µL of Hi-Di Formamide, 2.0 µL of SAP purified SNaPshot product and 0.1 µL of GeneScan-120 LIZ internal size standard (Applied Biosystems) was denatured at 96 °C for 5 min and subjected to capillary electrophoresis. Genotypes of the SNPs were identified by different fluorescent signals using ABI-3130XL Genetic Analyzer and Genemapper ID-X software (Applied Biosystems).

Table 1. Demographic data of study participants.

Variable	Case	Control	p Value
N	101	102	NA
Sex, female (%age; SD)	58 (57.42%; 0.497)	32 (31.37%; 0.466)	0.000
Mean age (SD)	21.59 (15.6)	30.15 (9.1)	0.076
Positive family history	68.53%	0%	NA

Table 2. Sequence of primers used to amplify genomic regions of target ADAM33 SNPs.

Sr. no.	Target SNP	Primer	Sequence (5’–3’)	PCR product length (bp)
1	S2 [rs528557]	F	AGAGCTCTGAGGAGGGGAAC	208
		R	GACCATGACACCTTCCTGCT	
2	ST + 5 [rs597980]	F	GCCACTTCCTCTGCACAAAT	157
		R	ACCACCCAGGTCACAGAGAA	
3	F + 1 [rs511898]	F	CCAGCTCCCAGAAGGAAGTT	243
		R	CTGCTCACCTGGAAAGGAAC	
4	ST + 4 [rs44707]	F	TTTTTCCCTGGCTCAGATTG	359
		R	TCCCAAGACCAGGCTATGTC	
5	T1 [rs2280091]	F	GGTGGAGGGTAAATGTTGTGA	397
		R	AGAGGGTGACTTGGAGCAGA	
6	T2 [rs2280090]	F	CTGCTGGGCTCATGAGAGTT	442
		R	GAGCTGAGAGGGTGACTTGG	
7	T + 1 [rs2280089]	F	CTGAGCCCAGAAACCTGATT	483
		R	GATCACTGTGCCAACCTCCT	
8	Q – 1 [rs612709]	F	AGCCTGGCTTGTCACAGAAG	588
		R	CTGAGTGAGGGGAGCAGAAG	

Sr., serial; F, forward; R, reverse.

Table 3. Sequence of single base extension primers.

Target SNP	Primer orientation	SNP	SBE primer sequence*	Primer length (bp)
S2[rs528557]	REVERSE	G/C	GACTGACTGACTGACTGACTCTGCCTCTGCTCCCAGG	37
ST + 5[rs597980]	REVERSE	T/C	GACTGACTGACTGACTGACTGACTGACTGGGCAGCCAGCACC	42
F + 1[rs511898]	REVERSE	T/C	GACTGACTGACTGACTGACTGACTGAGCCCTCCAAATCAGAAGAGAC	47
ST + 4[rs44707]	FORWARD	A/C	GACTGACTGACTGACTGACTGACTGACTGACCACAAATCACCTCTGTCACCC	52
T1[rs2280091]	FORWARD	G/A	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACT TGTGGGGCCCAACTCC	56
T2[rs2280090]	REVERSE	A/G	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACT CCCACAGCCACTGGACAG	70
T + 1[rs2280089]	REVERSE	G/A	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACT GCTCATGCCTCCTGCCT	60
Q – 1[rs612709]	REVERSE	G/A	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACT GCTCTGCCCCACCCGAC	67

*SBE, single base extension; bold indicates poly (dGACT) tail.

Statistical analysis

The allele and genotype frequency distribution, the Chi-squared test, and association of genetic variants with asthma was performed using SHesis online software [40]. Haploview software v4.2 was used for calculating pairwise linkage disequilibrium (LD) among the SNPs [41]. LD block construction and Hardy–Weinberg Equilibrium (HWE) analysis were performed using data from control subjects only. SNPs with less than 90% genotyping call rate (i.e. those that failed to be genotyped in >10% of the samples) and which failed the HWE test were excluded from further analysis. The cutoff *p* value for HWE test was <0.001.

Results

Allelic and genotypic frequency distribution

The average age of patients and control subjects was 21.59 ± 15 and 30.15 ± 9.16 years, respectively. 43 of the 101 asthma patients were male and 58 were female; 70 of the 102 control subjects were males and 32 were female (Table 1). Peripheral blood samples were taken from all subjects and DNA was extracted. ADAM33 SNPs T + 1[rs2280089], T2[rs2280090], T1[rs2280091], ST + 5[rs597980], ST + 4[rs44707], S2[rs528557], Q – 1[rs612709], and F + 1[rs511898] were amplified in a multiplex PCR reaction, fluorescently labeled by SBE and separated by capillary electrophoresis. The multiplex amplification profile of an individual subject is shown in Figure 2.

Of the 8 SNPs successfully genotyped, only one, S2[rs528557], showed significant difference in allele and genotype frequency between asthma patients and control subjects (Allele *p* = 0.0189, Genotype *p* = 0.0217). There were no significant differences (*p* > 0.05) in either allele or genotype frequency between patients and controls for the remaining seven SNPs [T + 1, T2, T1, ST + 5, ST + 4, Q – 1, F + 1] (Table 4). No significant changes were found in associations of SNPs with asthma on taking positive family history of the disease as covariate.

Linkage disequilibrium and haplotypes

To evaluate potential LD between ADAM33 SNPs, pairwise normalized coefficient of disequilibrium (*D'* values) were calculated at 95% confidence interval using Haploview v4.2

software [42] and an LD block was constructed (Figure 3). Two of the 8 SNPs (ST + 5[rs597980], F + 1[rs511898]) were excluded from LD analysis because they did not pass the HWE test. LD analysis showed that the six SNPs are in moderate to strong LD in pairs.

The six SNPs in the LD block were analyzed at the haplotype frequency level using PLINK v1.9 software and verified with Haploview software v4.2 [41,42]. The purpose of haplotype analysis was to determine whether any combination of alleles is associated with the risk of asthma. As shown in Table 5, the frequency of haplotype H2 ‘‘AAGTGG’’ was higher in asthma patients (0.141) than in healthy controls (0.095), although not significantly higher (*p* = 0.15; Chi-square = 2.04), suggesting a trend toward association of this haplotype with risk of asthma. Interestingly, the frequency of haplotype ‘‘AAGTCG’’ was significantly higher in healthy controls than in asthma patients (*p* = 0.0059; Chi-square = 7.579) even after applying 1000 permutations (*p* = 0.023), suggesting that this haplotype has a protective effect against asthma.

Discussion

Over-expression of ADAM33 has been shown in mesenchymal, smooth muscle and epithelium cells from asthma patients compared to cells from healthy individuals [43]. Different domains of ADAM33 gene translate into different functions that somehow are closely related to other ADAM genes, whose protein products possess proteolytic activity which could play an important role in the airway remodeling in asthma [13,14]. The reported over expression of ADAM33 in asthma patients and association of ADAM33 SNPs with asthma in multiple populations favors the hypothesis that ADAM33 is involved in asthma susceptibility [43]. Genetic polymorphisms of ADAM33 have been shown to have variable impact on asthma risk in different populations [32]. In this case-control study, we observed significant association of one ADAM33 SNP, S2[rs528557], with risk of asthma in Punjabi subjects from Pakistan. The significant association of S2[rs528557] with asthma, under both allelic (*p* = 0.0189; CI = 1.439 [0.945–2.192]) and genotypic models (*p* = 0.0217; Chi-square = 7.659), in this study is consistent with results from previous studies of Thai [27], adult Indian [22], pediatric Indian [19], Japanese [23] and European subjects [36], and

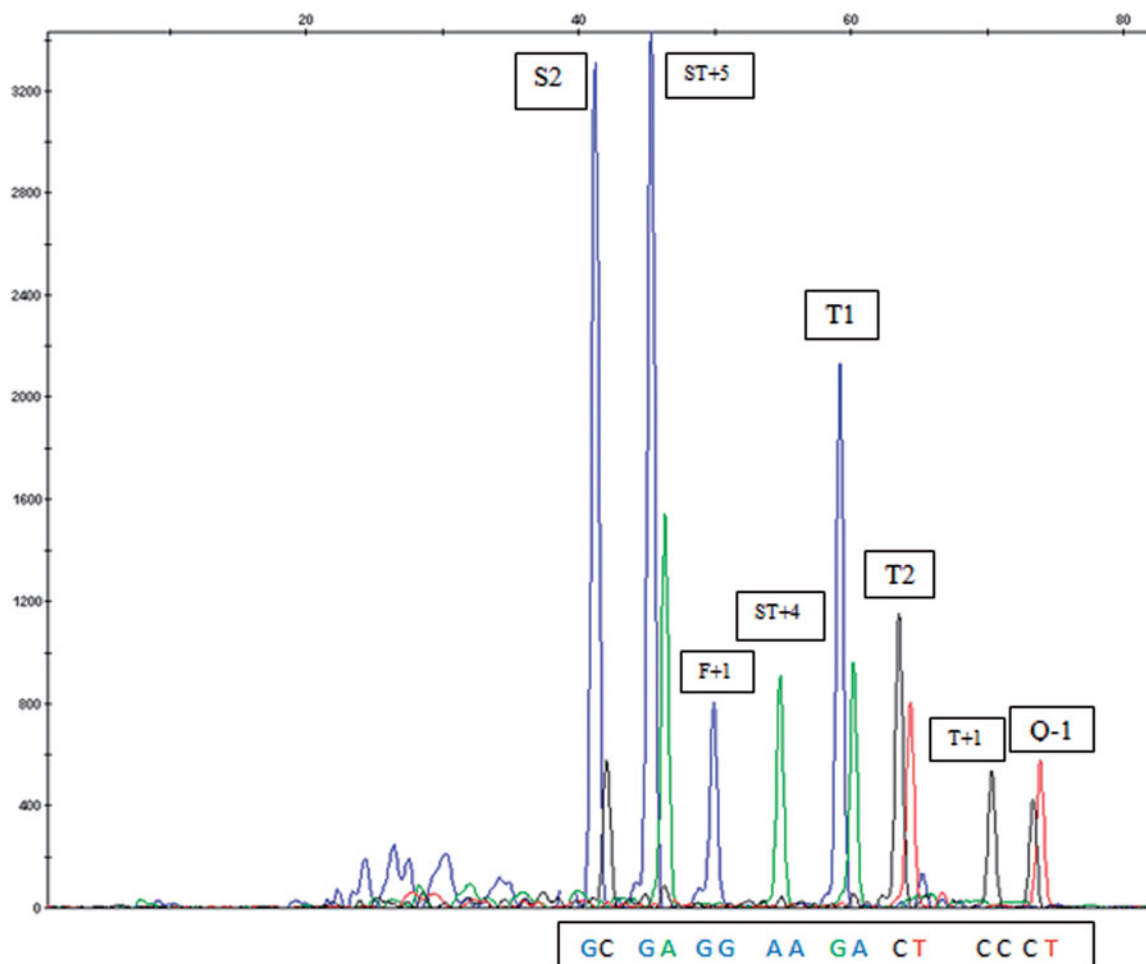


Figure 2. Representative multiplex SNaPSHOT profile of eight ADAM33 SNPs. This profile was generated by an ABI-3130XL genetic analyzer using GeneMapper IDx software. Labels on the peaks are the SNP IDs; labels below the peaks show the genotype of each SNP.

contrary to results from studies of east Han Chinese [29] and Asian subjects [39].

The SNP S2[rs528557] is located in the exonic region of ADAM33 that encodes the transmembrane domain, which possibly anchors the ADAM33 protein to cell membrane. Although this SNP encodes a synonymous exon (Gly717Gly), without altering amino acid sequence, it may influence mRNA folding, stability and translation, as has been shown for SNPs in other genes [44], and thereby alter ADAM33 function and favor asthma pathogenesis. Of the six ADAM33 SNPs found to be in moderate to strong LD in pairs, only S2[rs528557] showed association with asthma independently. However, all six SNPs might influence asthma pathogenesis by acting collectively. These findings were also confirmed by performing logistic regression taking positive family history as covariate. Only S2 was still found to be associated with asthma (i.e. $p=0.03$) while remaining SNPs showed non-significant associations as earlier (Table 4).

The haplotype H2 ‘‘AAGTGG’’, showed a trend toward association with asthma. This haplotype might influence asthma pathogenesis through the combination of the alleles of these SNPs. The haplotype H1 ‘‘AAGTCG’’ showed a statistically significant association with healthy controls relative to asthma patients even after applying 1000 permutations. Therefore this haplotype may have a protective effect against asthma.

Variants of some SNPs were more prevalent in asthma patients than healthy controls in this study, including allele ‘‘A’’ of ST + 5[rs597980], allele ‘‘A’’ and genotype ‘‘AA’’ of Q – 1[rs612709], and genotypes ‘‘TT’’ and ‘‘GG’’ of ST + 4[rs44707], but the associations of these SNPs were not statistically significant in the sampled population ($p>0.05$). Association of these ADAM33 SNPs with asthma seems to depend on the study population. Significant association of the ST + 5 ‘‘A’’ variant with asthma has been observed in Indians [20,23] and Germans [34,35], but a meta-analysis study reported contradictory results for the same variant in Asian populations [38,39]. In the SNP marker Q – 1[rs612709], allele ‘‘A’’ has been reported as being significantly correlated to the risk of asthma in Chinese Han [31], and Japanese women [25] but no association with asthma risk was found in northern Han Chinese [30] and Asian populations [39]. Association of ST + 4[rs444707] with asthma has been reported in Indian adults [22], Thai [27], Asian [36], and German populations [35]. No association of this variant with asthma was found in east Han Chinese [29], Asian [39], and Saudi children [18].

The non-significant differences in SNP frequencies between asthma patients and controls in the present study might be due to the relatively small sample size, or it could be that the influence of these SNPs on asthma susceptibility is very small in this study population. However, the significant

Table 4. Allelic and genotypic association tests of ADAM33 SNP variants on SHEsis and PLINK 1.9 [40,42].

SNP	Allele	Global MAF	Allele freq. cases	Allele freq. controls	Odds ratio [95%CI]	Pearson's <i>p</i> value	Odds ratio ^a [95%CI]	Pearson's <i>p</i> value ^b	Genotype	Genotype freq. patients	Genotype freq. controls	Chi ²	Pearson's <i>p</i> value
T + 1[rs2280089]	G	A = 0.136	0.851	0.847	0.9651 [0.5573~1.671]	0.8992	0.584 [0.21~1.61]	0.298	G/G	0.713	0.716	0.001	0.999
	A		0.149	0.153					G/A	0.277	0.275		
T2[rs2280090]	G	A = 0.146	0.842	0.851	1.079 [0.628~1.854]	0.782	NA	NA	A/A	0.010	0.010		
	A		0.158	0.154					G/G	0.693	0.713	0.097	0.952
T1[rs2280091]	A	G = 0.142	0.842	0.843	1.009 [0.596~1.711]	0.965	0.535 [0.19~1.48]	0.227	G/A	0.297	0.277		
	G		0.158	0.157					A/A	0.010	0.010		
ST + 5[rs597980]	A	A = 0.380	0.554	0.520	0.937 [0.662~1.325]	0.484	0.8948 [0.4173~1.919]	0.775	A/A	0.693	0.696	0.002	0.998
	G		0.446	0.480					A/G	0.297	0.294		
ST + 4[rs44707]	T	G = 0.424	0.703	0.701	0.990 [0.647~1.5156]	0.965	NA	NA	G/G	0.010	0.010	1.082	0.582
	G		0.297	0.299					A/A	0.238	0.178		
S2[rs528557]	G	G = 0.371	0.342	0.235	1.439 [0.945~2.192]	0.0189	2.319 [1.073~5.012]	0.032	A/G	0.634	0.683		
	C		0.658	0.765					G/G	0.129	0.139	1.996	0.368
Q – 1[rs612709]	G	A = 0.223	0.799	0.822	1.160 [0.701~1.918]	0.562	NA	NA	T/T	0.515	0.471		
	A		0.201	0.178					T/G	0.376	0.461		
F + 1[rs511898]	T	T = 0.455	0.347	0.327	1.0711 [0.726~1.579]	0.673	1.044 [0.4834~2.256]	0.912	G/G	0.109	0.069	7.659	0.0217
	C		0.653	0.673					G/C	0.582	0.450		
									C/C	0.367	0.540	1.198	0.549
									G/G	0.660	0.673		
									G/A	0.278	0.297		
									A/A	0.062	0.030		
									T/T	0.059	0.030	1.047	0.592
									T/C	0.574	0.594		
									C/C	0.366	0.376		

Freq., frequency; MAF, minor allele frequency;
^aOdds ratios for logistic regression taking family history of asthma as covariate;
^b*p* values calculated from logistic regression taking family history of asthma as covariate.

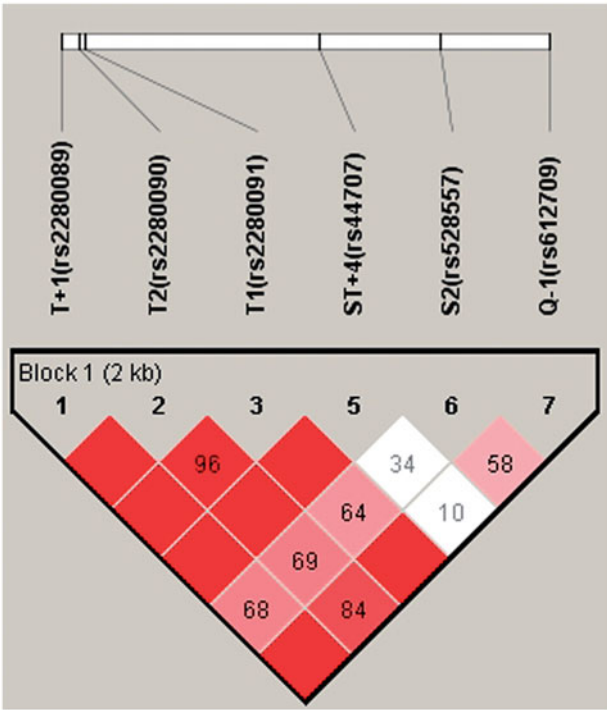


Figure 3. Linkage disequilibrium (LD) between six ADAM33 SNPs. LD block was generated using Haploview software. SNPs with genotyping call rates <90% were excluded. The relative physical position of each SNP is given at the top of the diagram. Numbers in the diamonds are D' values in percent. D' ≥ 80% = moderate LD; D' ≥ 90% = strong LD. Diamonds without numbers indicate D' = 100%.

Table 5. Haplotype analysis of ADAM33 SNP variants (T + 1, T2, T1, ST + 4, S2, and Q – 1) on Haploview v 4.2 [40].

Haplotype code	HAPLOTYPE	Freq. patients	Freq. controls	Chi-square	p Value	p Value ^a
H1	A A G T C G*	0.004	0.046	7.579	0.0059*	0.0230*
H2	A A G T G G	0.141	0.095	2.04	0.1532	0.6950
H3	G G A G C G	0.222	0.232	0.053	0.8174	1.0000
H4	G G A G G A	0.049	0.033	0.676	0.4109	0.9850
H5	G G A T C A	0.028	0.052	1.474	0.2247	0.8530
H6	G G A T C G	0.383	0.395	0.061	0.8045	1.0000
H7	G G A T G A	0.102	0.076	0.839	0.3598	0.9710

Freq., frequency;
*p value is significant (p<0.05);
^ap value is adjusted after applying 1000 permutations.

association of S2[rs528557] SNP and the possible trend of haplotype “AAGTGG” toward association, along with the protective role of haplotype “AAGTCG” against asthma, predicts the involvement of ADAM33 in asthma susceptibility in the Punjabi population of Pakistan.

Conclusions

This study demonstrated an association of ADAM33 gene variant S2[rs528557] with risk of asthma in the Punjabi population of Pakistan. Haplotype analysis revealed a trend toward association of haplotype “AAGTGG” with asthma risk, based on the difference in frequencies between patients and control groups. In addition, the haplotype “AAGTCG” was shown to occur with significantly higher frequency in the healthy control group, suggesting a protective effect against

asthma susceptibility. The small sample size may be a limitation of this study. Therefore, further studies of larger patient cohorts from Pakistan are needed to confirm these findings and improve understanding of the mechanism of ADAM33 involvement in asthma susceptibility.

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Declaration of interest

The authors declare no conflict of interest. The study was mainly sponsored by Centre for Applied Molecular Biology (CAMB), Lahore, Pakistan.

References

1. Cockcroft DW, Davis BE. Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006;118:551–559. Quiz 560–551.
2. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA dissemination committee report. *Allergy* 2004;59:469–478.
3. Cruz AA, Bousquet J, Khaltayev N. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. New York: World Health Organization; 2007.
4. Shahid M, Sabar MF, Bano I, Rahman Z, Iqbal Z, Ali SS, Ghani MU, et al. Sequence variants on 17q21 are associated with the susceptibility of asthma in the population of Lahore, Pakistan. *J Asthma* 2015;52:777–784.
5. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, Boulet LP. Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health* 2012; 12:204.
6. Selgrade MK, Lemanske Jr RF, Gilmour MI, Neas LM, Ward MD, Henneberger PK, Weissman DN, et al. Induction of asthma and the environment: what we know and need to know. *Environ Health Perspect* 2006;114:615–619.
7. Mustafa G, Khan PA, Iqbal I. Nocturnal asthma in school children of south Punjab, Pakistan. *J Ayub Med Coll* 2008;20:36–39.
8. Yusuf MO. Systems for the management of respiratory disease in primary care—an international series: Pakistan. *Prim Care Respir J* 2009;18:3–9.
9. Wan Y, Shrine N, Artigas MS, Wain L, Blakey J, Moffatt M, Bush A, et al. Genome-wide association study to identify genetic determinants of severe asthma. *Thorax* 2012;67:762–768.
10. Reinius LE, Gref A, Saaf A, Acevedo N, Joerink M, Kupczyk M, D’Amato M, et al. DNA methylation in the Neuropeptide S Receptor 1 (NPSR1) promoter in relation to asthma and environmental factors. *PLoS One* 2013;8:e53877.
11. Louis R, Schleich F, Corhay JL, Louis E. [Asthma: a complex disease determined by genetic and environmental factors]. *Rev Med Liege* 2012;67:286–291.
12. Rosenwasser LJ. Genetics of asthma and atopy. *Toxicol Lett* 1996; 86:73–77.
13. Foley SC, Mogas AK, Olivenstein R, Fiset PO, Chakir J, Bourbeau J, Ernst P, et al. Increased expression of ADAM33 and ADAM8 with disease progression in asthma. *J Allergy Clin Immunol* 2007;119:863–871.
14. Primakoff P, Myles DG. The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet* 2000;16:83–87.
15. Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma

- disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J* 2007;29:793–803.
16. Gao PS, Huang SK. Genetic aspects of asthma. *Panminerva Med* 2004;46:121–134.
 17. Bijanzadeh M, Mahesh PA, Ramachandra NB. An understanding of the genetic basis of asthma. *Indian J Med Res* 2011;134:149.
 18. Al-Khayyat AI, Al-Anazi M, Warsy A, Vazquez-Tello A, Alamri AM, Halwani R, Alangari A, et al. T1 and T2 ADAM33 single nucleotide polymorphisms and the risk of childhood asthma in a Saudi Arabian population: a pilot study. *Ann Saudi Med* 2012;32:479–486.
 19. Awasthi S, Tripathi P, Ganesh S, Husain N. Association of ADAM33 gene polymorphisms with asthma in Indian children. *J Hum Genet* 2011;56:188–195.
 20. Tripathi P, Awasthi S, Gao P. ADAM metalloproteinase domain 33 (ADAM33): a promising target for asthma. *Mediators Inflamm* 2014;2014:572025.
 21. Tripathi P, Awasthi S, Prasad R, Ganesh S. Haplotypic association of ADAM33 (T + 1, S + 1 and V - 3) gene variants in genetic susceptibility to asthma in Indian population. *Ann Hum Biol* 2012;39:479–483.
 22. Tripathi P, Awasthi S, Prasad R, Husain N, Ganesh S. Association of ADAM33 gene polymorphisms with adult-onset asthma and its severity in an Indian adult population. *J Genet* 2011;90:265–273.
 23. Hirota T, Hasegawa K, Obara K, Matsuda A, Akahoshi M, Nakashima K, Shirakawa T, et al. Association between ADAM33 polymorphisms and adult asthma in the Japanese population. *Clin Exp Allergy* 2006;36:884–891.
 24. Matsusue A, Kiyohara C, Tanaka K, Sasaki S, Miyake Y. ADAM33 genetic polymorphisms and risk of atopic dermatitis among Japanese children. *Clin Biochem* 2009;42:477–483.
 25. Miyake Y, Tanaka K, Arakawa M. ADAM33 polymorphisms, smoking and asthma in Japanese women: the Kyushu Okinawa maternal and child health study. *Int J Tuberc Lung Dis* 2012;16:974–979.
 26. Noguchi E, Ohtsuki Y, Tokunaga K, Yamaoka-Sageshima M, Ichikawa K, Aoki T, Shibasaki M, et al. ADAM33 polymorphisms are associated with asthma susceptibility in a Japanese population. *Clin Exp Allergy* 2006;36:602–608.
 27. Thongngarm T, Jameekornrak A, Limwongse C, Sangasapaviliya A, Jirapongsananuruk O, Assawamakin A, Chaiyaratana N, et al. Association between ADAM33 polymorphisms and asthma in a Thai population. *Asian Pac J Allergy Immunol* 2008;26:205–211.
 28. Lee JH, Park HS, Park SW, Jang AS, Uh ST, Rhim T, Park CS, et al. ADAM33 polymorphism: association with bronchial hyper-responsiveness in Korean asthmatics. *Clin Exp Allergy* 2004;34:860–865.
 29. Jie Z, Hu Z, Bai C, Jin M. ADAM33 gene polymorphisms associate with asthma susceptibility and severity in East China han population. *J Asthma* 2011;48:979–985.
 30. Qu S, Sun D, Wang Y, Zhang C, Lv Y, Yao L. Association of ADAM33 polymorphisms with childhood asthma in a northern Chinese population. *Exp Mol Pathol* 2011;91:775–779.
 31. Su D, Zhang X, Sui H, Lu F, Jin L, Zhang J. Association of ADAM33 gene polymorphisms with adult allergic asthma and rhinitis in a Chinese han population. *BMC Med Genet* 2008;9:82.
 32. Wang J, Wen J, Si-Ma-Yi MH, He YB, Tu-Er-Xun KL, Xia Y, Zhang JL, et al. Association of ADAM33 gene polymorphisms with asthma in the Uygur population of China. *Biomed Rep* 2013;1:447–453.
 33. Sadeghnejad A, Ohar JA, Zheng SL, Sterling DA, Hawkins GA, Meyers DA, Bleecker ER. ADAM33 polymorphisms are associated with COPD and lung function in long-term tobacco smokers. *Respir Res* 2009;10:21.
 34. Schedel M, Depner M, Schoen C, Weiland SK, Vogelberg C, Niggemann B, Lau S, et al. The role of polymorphisms in ADAM33, a disintegrin and metalloprotease 33, in childhood asthma and lung function in two German populations. *Respir Res* 2006;7:91.
 35. Werner M, Herbon N, Gohlke H, Altmüller J, Knapp M, Heinrich J, Wjst M. Asthma is associated with single-nucleotide polymorphisms in ADAM33. *Clin Exp Allergy* 2004;34:26–31.
 36. Song GG, Kim JH, Lee YH. Association between ADAM33 S2 and ST + 4 polymorphisms and susceptibility to asthma: a meta-analysis. *Gene* 2013;524:72–78.
 37. Raby BA, Silverman EK, Kwiatkowski DJ, Lange C, Lazarus R, Weiss ST. ADAM33 polymorphisms and phenotype associations in childhood asthma. *J Allergy Clin Immunol* 2004;113:1071–1078.
 38. Lee YH, Song GG. Association between ADAM33 T1 polymorphism and susceptibility to asthma in Asians. *Inflamm Res* 2012;61:1355–1362.
 39. Liang S, Wei X, Gong C, Wei J, Chen Z, Deng J. A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. *Hum Immunol* 2013;74:648–657.
 40. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005;15:97–98.
 41. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265.
 42. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575.
 43. Tripathi P, Awasthi S, Husain N, Prasad R, Mishra V. Increased expression of ADAM33 protein in asthmatic patients as compared to non-asthmatic controls. *Indian J Med Res* 2013;137:507–514.
 44. Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet* 2003;12:205–216.