RESEARCH ARTICLE

Association of *ADAM33* gene polymorphisms with adult-onset asthma and its severity in an Indian adult population

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Abstract

ADAM33, a member of the *ADAM* (a disintegrin and metalloprotease) gene family, is an asthma susceptibility gene originally identified by positional cloning. In the present study, we investigated the possible association of five single-nucleotide polymorphisms (SNPs) in the *ADAM33* (rs511898, rs528557, rs44707, rs597980 and rs2787094) with adult-onset asthma in an Indian population. The study included 175 patients with mild intermittent ($n = 44$), mild persistent ($n = 108$) or moderate persistent ($n = 23$) subgroups of asthma, and 253 nonasthmatic control individuals. SNPs were genotyped with the help of restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) method, and data were analysed using chi-square test and logistic regression model. Bonferroni's correction for multiple comparisons was applied for each hypothesis. Genotypes and allele frequencies of SNPs rs511898 and rs528557 were significantly associated with adult-onset asthma ($P = 0.010$-<0.001). A significant association of the homozygous mutant genotype and mutant alleles of SNPs rs2787094, rs44707 and rs597980 with the asthma was also observed ($P = 0.020$-<0.001). A positive association between asthma and haplotypes AGCCT, GGCCT, AGACT, GCAGT, GGACT, ACCCC and AGACC were also found ($P = 0.036$-<0.001, OR = 2.07–8.49). Haplotypes AGCGT, GCAGC, ACAGC, ACAGT, GGAGC and GGCGT appear to protect against asthma ($P = 0.013$-<0.0001, OR = 0.34–0.10). Our data suggest that ADAM33 gene polymorphisms serve as genetic risk factors for asthma in Indian adult population.

[Tripathi P., Awasthi S., Prasad R., Husain N. and Ganesh S. 2011 Association of *ADAM33* gene polymorphisms with adult-onset asthma and its severity in an Indian adult population. *J. Genet.* **90**, 265–273]

Introduction

Asthma is a complex disease thought to arise from interactions between multiple genes and several environmental factors (Newman-Taylor 1995; Hoffjan and Ober 2002; Illig and Wjst 2002; Palmer and Cookson 2002; Wenzel 2006). Asthma is an epidemic, affecting more than 155 million subjects worldwide (Hoffjan and Ober 2002), and its prevalence in India is about 2.38% (Aggarwal *et al.* 2006). Asthma is defined as 'a chronic inflammatory disorder of the conducting airways and is characterized by the airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or early morning' (Global Initiative for Asthma (GINA) guidelines 2005). A gene encoding a disintegrin and metalloprotease (*ADAM33*) located on chromosome 20p13 (figure 1) was the first identified as an asthma susceptibility gene by positional cloning approach in the year 2002 in a genomewide scan of a Caucasian population (Van Eerdewegh *et al.* 2002). *ADAM33* is a member of the multifunctional *ADAM* family of genes that code for zinc-dependent metalloproteases. The *ADAM33* protein harbours several domains which include prometalloprotease-like, disintegrin-like, cysteine-rich, epidermal growth factor-like, transmembrane, and cytoplasmic domains, and many specific functions of these domains have already been discovered (Yoshinaka *et al.* 2002). It is highly expressed in lung, heart, and brain of adults (Gunn *et al.* 2002). However, the specific process by which *ADAM33* variants could cause asthma is unknown. One of the hypotheses suggests that

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Keywords. polymorphisms; asthma; severity; haplotypes; linkage disequilibrium.

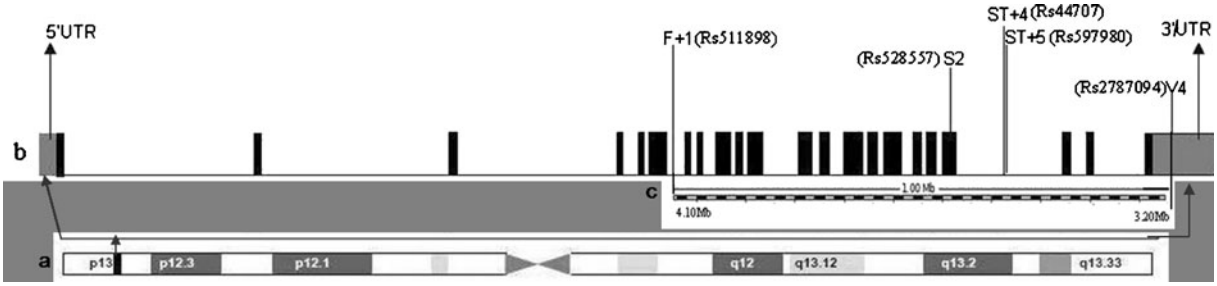

Figure 1. Schematic representation of *ADAM33* gene on chromosome 20. a) chromosome 20 showing *ADAM33* gene position 20p13. b) Position of the genotyped polymorphisms (SNPs) in the *ADAM33* gene in respect to the 22 exons, introns and untranslated regions of the gene. c) Region covered by five genotyped polymorphisms (SNPs) in Mb (megabase).

ADAM33 may be involved in airway remodelling (Shapiro and Owen 2002). The *ADAM33* is indeed expressed in airway fibroblasts, myofibroblasts, and smooth muscle (Van Eerdewegh *et al.* 2002). An association with *ADAM33* variants in adult-onset asthma has been observed in several populations (Howard *et al.* 2003; Lind *et al.* 2003; Lee *et al.* 2004; Raby *et al.* 2004; Werner *et al.* 2004; Blakey *et al.* 2005; Hirota *et al.* 2006; Kedda *et al.* 2006; Noguchi *et al.* 2006; Schedel *et al.* 2006; Wang *et al.* 2006; Sakagami *et al.* 2007; Su *et al.* 2008; Thongngarm *et al.* 2008; Bijanzadeh *et al.* 2010; Vergara *et al.* 2010). However, most such studies on *ADAM33* in asthma have been carried out on Caucasian populations; very few reports on *ADAM33* SNPs in asthma are available for Asian populations (Lee *et al.* 2004; Hirota *et al.* 2006; Noguchi *et al.* 2006; Wang *et al.* 2006; Sakagami *et al.* 2007; Su *et al.* 2008; Bijanzadeh *et al.* 2010). We recently found a positive association for *ADAM33* polymorphism in the asthma affecting the children in a north Indian population (Awasthi *et al.* 2011). In this study, we examined whether such an association exists for the adult-onset asthma as well.

Material and methods

Subjects

The study group consisted of 175 cases and 253 controls. They were recruited for the study between August 2007 and September 2009, at the Department of Pulmonary Medicine, Chattrapati Shahuji Maharaj Medical University, Lucknow, India. The study design and protocol were approved by the institutional ethics committee and written informed consent was obtained from subjects. The diagnosis of asthma was based on the following inclusion criteria: (i) more than two or three episodes of wheezing and shortness of breath during the past year; (ii) diagnosis of asthma according to the treating physician together with the demonstration of reversible and variable airflow obstruction by spirometry; (iii) symptoms; and (iv) use of medications for asthma. The diagnosis and classification of the asthma severity was made according to Global Initiative for Asthma (GINA) guidelines (2005). Asthmatic patients were grouped either into one of the following three categories: (i) mild intermittent ($n = 44$); (ii) mild persistent ($n = 108$); (ii) moderate persistent ($n = 23$) and (iii) severe persistent (no such cases). The inclusion criteria for controls were: (i) no past or present physician's diagnosis of asthma and other pulmonary diseases; (ii) no history of wheezing, shortness of breath, and other symptoms of allergic diseases such as nasal and skin symptoms; (iii) no use of medications for asthma; and (iv) absence of first-degree relatives with a history of asthma. All subjects including cases and controls were personally interviewed about their age, medical history of other diseases, demographic features, family history of asthma, present smoking habit and number or *bidis*/cigarettes smoked per day, residence near heavy traffic/busy road with vehicular traffic and presence of industrial area near residence.

DNA extraction and genotyping

DNA was extracted from the peripheral blood leaucocytes using a commercially available DNA extraction kit as detailed in our recent study (Awasthi *et al.* 2011). Five SNPs rs511898, rs2787094, rs44707, rs528557 and rs597980 of the *ADAM33* were selected for screening (accessed through Entrez SNP database: http://www.ncbi.nlm.nih.gov/snp/). Genotyping was done by polymerase chain reaction, restriction fragment length polymorphisms (PCR-RFLP) (see figure 1 in the electronic supplementary material at http://www.ias.ernet/jgenet). PCR amplification was conducted in a total volume of 15 μL with 20 pmol of each primer. PCR conditions were as follows: initial denaturation of 95°C for 5 min, 40 cycles of 95°C for 45 s, 60–65°C for 45 s, and 72°C for 30 s, followed by a final extension of 72°C for 30 s. Detailed information on the location of SNPs, PCR conditions, primer sequences, restriction enzymes, etc. are summarized in table 1. The PCR products were then digested overnight by restriction endonuclease at 37°C and were thereafter separated by electrophoresis on 10% polyacrylamide gel and stained with ethidium bromide. Nearly 20% of samples were regenotyped and reproducibility was established.

Table 1. Description of the investigated ADAM33 SNPs.

SNP	SNP name	Location	alleles	Forward (F) and reverse (R) primers for PCR	Annealing temp. °C/extension time in sec per number of cycles	Digest (bp)/ enzyme
srs511898	F+1	Intron 6	G>A	(F)5′GTATCTATAGCCCTCCAAATCAGAAGAGCC-3′	60/30/40	166(A) *Msp*I
				(R)5′GGACCCTGAGTGGAAGCTG-3′		29+137(G)
rs2787094	V4	3′UTR	C>G	(F)5′CTCAGGAACCACCTAGGGGAGAAG3′	60/30/40	290(C) *Pst*I
				(R)5′CAAAGGTCACACAGCCCCTGACCT3′		196+94(G)
rs44707	ST+4	Intron 19	A>C	(F)5′CACTTCCTCTGCACAAATCACCTCTGTCGTC-3′	64/30/40	277 (C) *Hpy*166II
				(R)5′GAGCACTCCCAAGACCAGGCTATGTCAG-3′		246+31(A)
rs528557	S2	Exon 19	G>C	(F)5′AGAGCTCTGAGGAGGGGAACCG-3′	64/30/40	211(C) *Nar*I
				(R)5′GCAGACCATGACACCTTCCTGCTG-3′		147+64(G)
rs597980	ST+5	Intron 19	C>T	(F)5′TCCCTGGCTCAGATTGCAGTCC-3′	65/30/40	239(T) *Acl*I
				(R)5′ACCACCCAGGTCACAGAGAACTGG-3′		177+62(C)

Statistical analysis

EpiINfo 6 (Atlanta, USA, http://www.cdc.gov/epo/epi/epiinfo.htm) and SPSS 11.5 (Chicago, USA) statistical tools were used for the statistical analysis. 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. Chi square (χ^2) test was used to determine differences in genotype/allele frequencies and deviation from Hardy–Weinberg equilibrium (HWE). HWE for the cases and controls were tested separately. Binary logistic regression was used to calculate odds ratio (OR). Homozygous genotype for the normal allele of each SNP in the control group was used as reference in calculating OR and 95% confidence intervals (CI) as reported (Upadhyay *et al.* 2009). P value was considered significant at $P < 0.05$ level. P values were corrected (Pcorr) for multiple corrections (Bonferroni correction) (He *et al.* 2007; Upadhyay *et al.* 2009). Fisher's exact test was performed to avoid type one error in subgroup analyses having statistically low-powered sample size. Arlequin software version 2.00 (Schneider *et al.* 2000) and SNPAnalyzer version 1.0 (ISTECH, Kyungkido, Korea) were used to calculate the distribution of haplotypes and pairwise linkage disequilibrium between each pair of SNP loci that was evaluated by using an expectation–maximization algorithm (Schneider *et al.* 2000).

Results

We have included 175 cases of asthma of which 44 (25.1%) had mild intermittent, 108 (61.7%) had mild persistent and 23 (13.1%) had moderate persistent subgroup of asthma. None of the recruited patients showed severe persistent subgroup of asthma as per the criterion of the Global Initiative for Asthma (GINA) guidelines (2005). Table 2 details the clinical features of the cases analysed. As compared to the control group, cases had significantly higher number of smokers (OR = 3.33, 95% CI (2.16–5.15) P = <0.0001) and reside near industrial area (OR = 2.97, 95% CI (1.31–6.03) $P = 0.003$). Among the subgroups, the mild persistent asthmatic patients had more smokers than (OR = 2.45, 95% CI (1.12–5.39) $P = 0.014$) patients suffering from moderate persistent subgroup of asthma (OR = 1.60, 95% CI (0.51–5.06) $P = 0.364$). Hospitalization rate and family history for asthma were higher with patient suffering from moderate persistent compared with mild persistent subgroup of asthma.

Association of ADAM33 polymorphisms with asthma compared with controls

The genotype and allele frequencies of the *ADAM33* polymorphisms in patients and healthy controls are provided in table 3. To rule out any sampling bias, the HWE was calculated for both cases and control groups. All five SNPs genotyped were in HWE ($P > 0.05$) both in the cases and

Table 2. Demographic profile of selected population.

Basic demographic	Controls ($n = 253$)	Total cases ($n = 175$)	Mild intermittent $n = 44$ (25.1%)	Mild persistent $n = 108$ (61.7%)	Moderate persistent $n = 23$ (13.1%)
Gender (female) (n%)	52 (20.6)	54 (30.9)	10 (22.7)	36 (33.3)	8 (34.8)
Age (in years) (mean±SD)	31.9 ± 9.2	33.7 ± 11.3	31.31 ± 8.9	32.7 ± 11.4	42.5 ± 11.4
Weight (in kg) (mean±SD)	61.9 ± 10.4	58.6 ± 11.9	57.4 ± 10.6	57.9 ± 11.5	62.13 ± 15.39
Height (in cm) (mean±SD)	164.2 ± 7.9	161.4 ± 7.3	162.4 ± 6.6	161.2 ± 7.1	160.4 ± 9.42
BMI (mean±SD)	22.3 ± 4.1	22.9 ± 3.5	21.7 ± 3.6	22.2 ± 3.8	24.1 ± 5.5
Presence of industrial factor	13 (5.1)	23 (13.1)*[1]	7 (15.9)	16 (14.8)	0
Type of road					
a. Road of occasional traffic	200 (79.1)	122 (69.7)	37 (84.1)	74 (68.5)	11 (47.8)
b. Busy road with vehicular traffic	53 (20.9)	37 (21.1)	6 (13.6)	23 (21.3)	8 (34.8)*[2]
	0 (0.0)	16 (9.1)	1 (2.3)	11 (10.2)	4 (17.4)
c. Near highway	0 (0.0)	16 (9.1)	1 (2.3)	11 (10.2)	4 (17.4)
Smoking status of subject					
a. Nonsmoker	192 (75.9)	85 (48.6)	28 (63.6)	45 (41.7)	12 (52.2)
b. Total smoker	61 (24.1)	90 (51.4)*[3]	16 (36.4)	63 (58.3)*[4]	11 (47.8)
I. One to two cigarettes or bidis per day	29 (11.5)	23 (13.1)	9 (20.5)	11 (10.2)	3 (13.0)
II. Three to ten cigarettes or bidis per days	11 (4.3)	31 (17.7)	6 (13.6)	21 (19.4)	4 (17.4)
III. More than 10 cigarettes or bidis per day	21 (8.3)	36 (20.6)	1 (2.3)	31 (28.7)	4 (17.4)
Hospitalization for asthma					
a. Present	–	3 (1.7)	–	2 (1.9)[NC]	1 (4.3)[NC]
b. In past	–	45 (25.7)	3 (6.8)	29 (26.9)*[5]	13 (56.5)*[6]
c. Total	–	45 (25.7)	3 (6.8)	29 (26.9)*[5]	13 (56.5)*[6]
Family history of asthma		84 (48.0)	21 (47.7)	50 (46.3)	13 (56.5)

BMI, body mass index n, number; SD, standard deviation; NC, not calculated; *[1], OR 2.97, 95% CI (1.31–6.03) $P = 0.003$ (controls versus cases); *[2], OR 3.38, 95% CI (0.86–13.55) $P = 0.043$ (mild intermittent versus moderate persistent); *[3], OR 3.33, 95% CI (2.16–5.15) $P = <0.0001$ (controls versus cases); *[4], OR 2.45, 95% CI (1.12–5.39) $P = 0.014$ (mild intermittent versus mild persistent); *[5], OR 5.02, 95% CI (1.35–22.04) $P = <0.006$ (mild intermittent versus mild persistent); *[6], OR 17.77, 95% CI (3.66–98.74) $P = <0.0001$ (mild intermittent versus moderate persistent).

Table 3. Association of five genotyped *ADAM33* gene polymorphisms with asthma.

SNPs		Patients ($n = 175$)		Control ($n = 253$)		OR (95% CI) *P* value
		(*N*)	(%)	(*N*)	(%)	
rs511898 (G>A)	Genotypes					
	GG	25	14.3	61	24.1	1 (Reference)
	GA	77	44.0	116	45.8	2.0 (1.2–3.4) 0.010*[1]
	AA	73	41.7	76	30.0	3.6 (2.1–6.4) <0.001
	Alleles					
	G	127	36.3	268	53.0	1 (Reference)
	A	223	63.7	238	47.0	1.98 (1.5–2.6) <0.001
rs2787094 (C>G)	Genotypes					
	CC	17	9.7	47	18.6	1 (Reference)
	CG	70	40.0	133	52.6	1.5 (0.78–2.7) 0.240
	GG	88	50.3	73	28.9	3.3 (1.8–6.3) <0.001
	Alleles					
	C	104	29.7	227	44.9	1 (Reference)
	G	246	70.3	279	55.1	1.93 (1.4–2.6) <0.001
rs44707 (A>C)	Genotypes					
	AA	31	17.7	63	24.9	1 (Reference)
	AC	78	44.6	120	47.4	1.3 (0.79–2.2) 0.290
	CC	66	37.7	70	27.7	1.9 (1.1–3.3) 0.020*[2]
	Alleles					
	A	140	40.0	246	48.6	1 (Reference)
	C	210	60.0	260	51.4	1.42 (1.1–1.9) 0.013*[3]
rs528557 (G>C)	Genotypes					
	GG	20	11.4	159	62.8	1 (Reference)
	GC	67	38.3	79	31.2	6.7 (3.8–11.9) <0.0001
	CC	88	50.3	15	5.9	46.6 (22.7–95.7) <0.0001
	Alleles					
	G	107	30.6	397	78.5	1 (Reference)
	C	243	69.4	109	21.5	8.27 (6.1–11.3) <0.0001
rs597980 (C>T)	Genotypes					
	CC	18	10.3	65	25.7	1 (Reference)
	CT	61	34.9	129	51.0	1.7 (0.93–3.1) 0.083
	TT	96	54.9	59	23.3	5.9 (3.2–10.9) <0.001
	Alleles					
	C	97	27.7	259	51.2	1 (Reference)
	T	253	72.3	247	48.8	2.75 (2.1–3.7) <0.001

N, number; OR, odds ratio; CI, confidence interval. *After Bonferroni correction *P* corr, *[1], 0.03; *[2], 0.06; *[3], 0.039.

control cohorts. An increased risk for asthma was observed for the heterozygous genotype, homozygous mutant genotype, and mutant allele of the SNPs rs511898 (GA, AA and A respectively) and rs528557 (GC, CC and C respectively). An increased risk of asthma was observed for the homozygous mutant genotype and mutant allele of the SNPs rs2787094 (GG and G respectively), rs44707(CC and C respectively) and rs597980(TT and T respectively). However, after Bonferroni's correction, homozygous mutant genotype of SNP rs44707 (CC) was nonsignificant. No positive association with asthma was observed for the heterozygous genotypes of the SNPs rs597980, rs2787094 and rs44707 (CT, CG and AC respectively).

ADAM33 polymorphisms in patients with and without a family history of asthma

To test a possible difference the genotype/allele distribution pattern in patients with a family history of asthma and those without a family history of asthma, a 'case only' analysis was performed. We found that the homozygous mutant genotype (TT) of SNP rs597980 was marginally associated with patients with a family history of asthma (OR = 1.74, 95% CI (0.91–3.32) $P = 0.071$). Heterozygous genotype of SNP rs597980 and the mutant and heterozygous genotypes of rest of the SNPs did not show an association with patients that had a family history of asthma. (see table 1 of supplementary material).

Association of ADAM33 polymorphisms with severity of asthma

The genotype and allele frequencies of the *ADAM33* polymorphisms with severity subgroups of asthma are provided in table 4. Here, we compared each subgroup of asthma with control group separately. Results showed that individuals with homozygous mutant genotype (AA) and heterozygous genotype (GA) of SNP rs511898, homozygous mutant genotype (CC) and heterozygous genotype (GC) of

Table 4. Association of *ADAM33* gene polymorphisms with severity of asthma.

	Control	Mild intermittent $n = 44$ (25.1%)		Mild persistent $n = 108$ (61.7%)		Moderate persistent $n = 23$ (13.1%)	
Genotype	*N* (%)	*N* (%)	OR (95%)	*N* (%)	OR (95%)	*N* (%)	OR (95%)
rs511898 (G>A)							
GG	76 (30.0)	4 (9.1)	1 (Reference)	19 (17.6)	1 (Reference)	2 (8.7)	1 (Reference)
GA	116 (45.8)	25 (56.8)	4.1 (1.4–12.2) 0.012*[1]	43 (39.8)	1.5 (0.80–2.7) 0.208	9 (39.1)	2.9 (0.62–14.0) 0.174
AA	61 (24.1)	15 (34.1)	4.7 (1.5–14.8) 0.009	46 (42.6)	3.01 (1.6–5.7) 0.001	12 (52.2)	7.5 (1.6–34.7) 0.010*[3]
rs2787094 (C>G)							
CC	47 (18.6)	4 (9.1)	1 (Reference)	11 (1.2)	1 (Reference)	2 (8.7)	1 (Reference)
CG	133 (52.6)	17 (38.6)	1.5 (0.48–4.7) 0.484	48 (44.4)	1.5 (0.74–3.2) 0.248	5 (21.7)	0.88 (0.17–4.7) 0.885
GG	73 (28.9)	23 (52.3)	3.7 (1.2–11.4) 0.22	49 (45.4)	2.9 (1.4–6.1) 0.006	16 (69.6)	5.2 (1.1–23.4) 0.034*[4]
rs44707 (A>C)							
AA	63 (24.9)	8 (18.2)	1 (Reference)	20 (18.5)	1 (Reference)	3 (13.0)	1 (Reference)
AC	120 (47.4)	19 (43.2)	1.2 (0.52–3.0) 0.623	52 (48.1)	1.4 (0.75–2.5) 0.309	7 (30.4)	1.2 (0.31–4.9) 0.774
CC	70 (27.7)	17 (38.6)	1.9 (0.77–4.7) 0.161	36 (33.3)	1.6 (0.85–3.1) 0.142	13 (56.5)	3.9 (1.1–14.3) 0.040*[5]
rs528557 (G>C)							
GG	159 (62.8)	4 (9.1)	1 (Reference)	15 (13.9)	1 (Reference)	1 (4.3)	1 (Reference)
GC	79 (31.2)	16 (36.4)	8.5 (2.6–24.90) <0.0001	44 (40.7)	5.9 (3.1–11.3) <0.001	7 (30.4)	14.1 (1.7–116.5) 0.014*[6]
CC	15 (5.9)	24 (54.5)	63.6 (19.5–207.7) <0.0001	49 (45.4)	34.6 (15.8–75.8) <0.0001	15 (65.2)	159.0 (19.6–1288.5) <0.0001
rs597980 (C>T)							
CC	65 (25.7)	5 (11.4)	1 (Reference)	11 (10.2)	1 (Reference)	2 (8.7)	1 (Reference)
CT	129 (51.0)	13 (29.5)	1.3 (0.45–3.8) 0.622	45 (41.7)	2.1 (1.0–4.3) 0.050*[2]	3 (13.0)	0.76 (0.12–4.6) 0.762
TT	59 (23.3)	26 (59.1)	5.7 (2.1–15.8) 0.001	52 (48.1)	5.2 (2.5–10.9) <0.001	18 (78.3)	9.9 (2.2–44.6) 0.003

N, number; OR, odds ratio; CI, confidence interval. *After Bonferroni correction *P* corr, *[1], 0.036; *[2], 0.15; *[3], 0.03; *[4], 0.102; *[5], 0.12; *[6], 0.042.

Table 5. Distribution of genotyped *ADAM33* gene polymorphism's haplotypes and its association with risk of asthma.

Haplotype							
(1)	(2)	(3)	(4)	(5)	Case ($n^a = 350$)	Control ($n^a = 506$)	OR (95% CI) *P* value
A	**G**	**C**	**C**	**T**	26.9	4.1	8.49 (5.01–14.49) <0.0001
G	G	**C**	**C**	**T**	9.5	2.4	4.26 (2.06–8.95) <0.0001
A	**G**	A	**C**	**T**	6.2	1.4	4.75 (1.88–12.52) 0.0001
G	C	A	G	**T**	5.9	2.9	2.07 (0.99–4.35) 0.0361
G	**G**	A	**C**	**T**	5.6	2.1	2.96 (1.27–6.97) 0.005
A	**G**	**C**	G	**T**	4.5	9.6	0.47 (0.25–0.88) 0.011
A	C	**C**	**C**	C	3.7	0.7	4.89 (1.45–18.12) 0.002
A	**G**	A	**C**	C	3.1	0.7	5.41 (1.37–24.94) 0.004
A	C	**C**	**C**	**T**	3.1	1.5	2.30 (0.80–6.76) 0.086
G	C	A	**C**	**T**	2.9	-	NC
A	**G**	**C**	G	C	2.8	2.3	1.19 (0.46–3.07) 0.695
G	C	A	G	C	2.8	10.3	0.25 (0.11–0.54) <0.0001
G	C	**C**	G	C	2.8	4.1	0.67 (0.28–1.56) 0.317
A	C	A	G	C	2.8	8.2	0.33 (0.15–0.71) 0.002
A	**G**	**C**	**C**	C	2.7	1.5	1.80 (0.63–5.18) 0.224
A	C	A	G	**T**	1.9	5.3	0.34 (0.12–0.87) 0.013
A	**G**	A	G	**T**	1.9	2.3	0.86 (0.28–2.54) 0.768
A	C	A	**C**	C	1.6	1.2	1.32 (0.35–4.93) 0.645
G	**G**	A	**C**	C	1.5	0.6	2.66 (0.55–14.12) 0.165
G	**G**	A	G	C	1.3	4.3	0.28 (0.08–0.86) 0.013
A	C	A	**C**	**T**	1.3	0.8	1.59 (0.33–7.59) 0.511
G	**G**	**C**	**C**	C	1.2	1.6	0.79 (0.20–2.91) 0.698
G	**G**	**C**	G	**T**	1.0	8.8	0.10 (0.02–0.33) <0.0001

n, Number; OR, odds ratio; CI, confidence interval. [a]Total number of chromosomes; NC, not calculated. (1) rs511898 (G>A); (2) rs2787094 (C>G); (3) rs44707 (A>C); (4) rs528557 (G>C); (5) rs597980 (C>T). *Mutant alleles are in bold font.

SNP rs528557 and homozygous mutant genotype (CC) of SNP rs597980 were at risk for mild intermittent subgroup of asthma. SNPs rs2787094 and rs44707 were not significantly associated with mild intermittent subgroup of asthma. Individuals with homozygous mutant genotypes of SNP rs511898 and rs2787094 (AA and GG respectively), heterozygous and homozygous mutant genotypes of SNPs rs528557 and rs597980 (GC/CC and CT/TT respectively) were at increased risk for mild persistent subgroup of asthma. However, after Bonferroni's correction, the heterozygous genotype (CT) of SNP rs597980 failed to show any association with the mild persistent subgroup of asthma. Genotypes of SNP rs44707 did not show an association with mild persistent subgroup of asthma. Individuals with homozygous mutant genotype of SNPs rs511898, rs2787094, rs44707 and rs597980 (AA, GG, CC and TT respectively) and heterozygous and homozygous mutant genotypes of SNP rs528557 (GC and CC) were at risk for moderate persistent subgroup of asthma. Nevertheless, after applying Bonferroni's correction, the homozygous mutant genotypes of SNPs rs2787094 and rs44707 were found to be 'nonsignificant'.

Significant results were observed with subgroups of asthma. However, results were conflicting to find out its association, based on increasing severity of asthma as per the criterion of the Global Initiative for Asthma (GINA) guidelines (2005). Mutant genotypes of SNPs rs511898, rs2787094, and rs597980 (AA, GG and TT respectively) were found to be more likely significantly associated with mild persistent subgroup of asthma as compared with moderate persistent. Likewise, heterozygous genotype of SNP rs597980 was highly associated with mild intermittent as compared to its associations with mild persistent subgroup and moderate persistent subgroups of asthma. This indicates that association was not related to increasing severity of asthma.

Association of ADAM33 haplotypes with risk of asthma

We constructed the ADAM33 haplotypes of cases and controls and evaluated their possible role in asthma. Haplotypes with frequencies >1% were selected for analysis (table 5). The frequency of haplotypes AGCCT, GGCCT, AGACT, GCAGT, GGACT, ACCCC and AGACC were significantly higher in cases than in controls ($P = 0.036$- <0.001, OR = 2.07–8.49). In contrast, frequency of haplotypes AGCGT, GCAGC, ACAGC, ACAGT, GGAGC and GGCGT was more common in the control group than in the case group ($P = 0.013$- <0.0001, OR = 0.34–0.10). We have also evaluated the pairwise linkage disequilibrium (LD) between each pair of SNP loci. Both for cases and controls, the LD measure D′ was high for all but one SNP tested in cases, and for two SNPs in the control group (Yates's corrected *P* value < 0.05 and $|D'| \neq 0$) (see table 2 in electronic supplementary material).

Discussion

In this case–control study, we analysed the association of five SNPs of spanning *ADAM33* and their haplotypes with the adult-onset asthma and its severity in an Indian population. We found that the homozygous mutant, heterozygous genotypes and allele frequencies of SNPs rs511898 and rs528557 located within the *ADAM33* gene were significantly associated with susceptibility to asthma. Further homozygous mutant genotypes and mutant alleles of SNPs rs2787094 and rs597980 also showed significant association with asthma. Since multiple SNPs may act together and increase the risk of asthma, haplotypes for five SNPs were constructed and their frequency were compared among cases and controls. Our haplotype analyses suggest that the haplotypes AGCCT, GGCCT, AGACT, GCAGT, GGACT, might serve as risk factors for asthma in the Indian population. Conversely, haplotypes AGCGT, GCAGC, ACAGC, ACAGT, GGAGC and GGCGT might serve as protective factors for the disease. Our results extend the previous findings on possible role of *ADAM33* in genetic susceptibility to asthma in diverse populations (Howard *et al*. 2003; Werner *et al.* 2004; Hirota *et al.* 2006; Noguchi *et al.* 2006; Su *et al.* 2008; Thongngarm *et al.* 2008). Lack of association for *ADAM33* gene in asthma have also been reported in populations from Korea (Lee *et al.* 2004), Australia (Kedda *et al.* 2006), P. R. China (Wang *et al.* 2006), Germany (Schedel *et al.* 2006), and the Caribbean coast of Colombia (Vergara *et al.* 2010). Intriguingly, the sets of SNPs have also shown significant association with the child-onset asthma in our recent study (Awasthi *et al.* 2011) suggesting a role for this gene both in childhood-onset and adulthood-onset asthma in the Indian population. Possible role for *ADAM33* gene in the childhood asthma have been excluded in an Mexican population (Lind *et al.* 2003) and in a Caucasian population of North America (Raby *et al.* 2004). Nonetheless, meta-analyses encompassing data from several studies reveal a positive association for the *ADAM33* SNPs rs511898 and rs1713853 with asthma. SNP rs1713853 was not tested in the present study (Blakey *et al.* 2005). Conversely, there seem to be a population specific variation with regard to the SNP(s) that show association with asthma.

However, SNP rs528557 was shown to be associated with asthma in a UK-based Caucasian population (Van Eerdewegh *et al.* 2002), a family-based study in Germans (Werner *et al.* 2004), in African-Americans, US whites, Hispanic (Howard *et al.* 2003), Japanese (Hirota *et al.* 2006), and in Thai population (Thongngarm *et al.* 2008), besides the present study on an Indian population. Similarly, SNP rs511898 was shown to associate with asthma in a UK and German populations (Van Eerdewegh *et al.* 2002; Werner *et al.* 2004) while the association of SNP rs597980 was restricted to the German families only (Werner *et al.* 2004). Intriguingly, SNP rs44707 was found to associate with asthma in diverse set of populations; US/UK combined, UK-only, Dutch white and in adult Chinese Han populations (Van Eerdewegh *et al.* 2002; Howard *et al.* 2003; Su *et al.* 2008). Thus, the present study adds to the growing list of population studies where the *ADAM33* SNPs seems to play a role in the susceptibility to asthma. These studies also highlight the fact that no single SNP is associated with asthma in all the populations studied. This may be due to the compound effects of multiple alleles, multiple genes and environmental factors since asthma is a multi-factorial disorder. For example, the effect of a given SNP could be restricted to an ethnic group or a population; thus, there may be genetic differences among subtypes of asthma, such as pediatric, adult, allergic/nonallergic. Secondly, this could be due to the difference in the exposure to allergens, which could vary from one geographical region to the other and their interactions with the genetic factor(s) could vary (Postma and Howard 2004; Vercelli 2004). Thirdly, the linkage disequilibrium patterns that exist between the identified SNP and the undetected causative defect in the gene could differ from one population to the other. Thus through understanding the genetic factors and their interactions with the environmental factors for each population would aid in developing effective predictive markers for the prevention and/or management of multifactorial diseases like asthma.

In summary, our results suggest an association between the *ADAM33* gene polymorphisms and asthma in the Indian population studied. Functional studies on the *ADAM33* SNPs and other sequence variants might uncover the molecular basis of susceptibility to asthma.

Acknowledgements

We acknowledge the financial support received from Department of Biotechnology, Government of India (BT/PR7115/Med/14/955/2006) for carrying out this work. We thank Dr Vikas Mishra and Ms Rashmi Parihar for the technical assistance. We also thank all the patients and healthy controls for providing blood samples.

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Received 8 November 2010, in final revised form 7 February 2011; accepted 15 February 2011
Published on the Web: 19 August 2011