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Original Article

Significant Association of ADAM33 V4C>G Polymorphisms with Asthma in a North Indian Population

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ABSTRACT: ADAM33 is zinc-dependent metalloproteases comprised of seven domains and has range of single nucleotide polymorphisms. These polymorphisms were found to be genetically associated in bronchial hyperresponsiveness that accounts its involvement in asthma. A case-control study was conducted with 483 healthy controls and 481 asthma patients in the present study. DNA samples were extracted from blood and the genotyping was done using PCR-RFLP method. Statistical analysis revealed that ADAM33 V4C>G polymorphism show highly significant association towards asthma with significant OR=1.40, p=0.017 in CG genotype and two fold risk in mutant GG genotype (p=0.000). Furthermore, mutant allele G was also highly significant towards the disease with OR=1.39 and p=0.000. However, ADAM33 F+1G>A, rs511898 gene polymorphism was significantly associated with asthma in only male and female phenotypes. This study concludes that the ADAM33 gene polymorphism plays an important role in the pathogenesis of asthma in a North Indian population.

KEYWORDS: ADAM33, asthma, case-control study, polymorphism, PCR-RFLP, total IgE.

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INTRODUCTION

Asthma is a multi-factorial disease, results from gene-environment interactions and genetic changes.[1] With the completion of the Human Genome Project, studies on single nucleotide polymorphisms (SNPs) have been explored to identify the genetic variants involved in the human diseases including asthma. These genetic variants are important in the pathogenesis as well as response to the treatment of the disease.[2]

ADAM33, a disintegrin and metalloprotease33, belongs to a family of 40 ADAM proteins and mainly expressed in mesenchymal cells in lung.[3] They are zinc-dependent metalloproteases that are type I transmembrane zymogen glycoproteins. It has been investigated that ADAM33 comprise of 22 exons that encode an 813 amino acids molecule with 7 domains.[4] In the past two decades, more than 300 SNPs of ADAM33 have been identified in humans and are found to be associated with asthma and allergic diseases.[5,6] However, the exact mechanism of ADAM33 in asthma pathogenesis is still not clear. They are expressed in lung mesenchymal cells and its genetic association with bronchial hyperresponsiveness (BHR) might be related with airway remolding in asthma.[7] They are also highly expressed in epithelium, myo/fibroblasts and airway smooth muscle cells which support their role in asthma pathogenesis. Furthermore, literature clearly shows that SNPs within the ADAM33 locus are associated with more rapid decline in lung function in asthmatics.[8-10]

The human ADAM33 gene is located on chromosome 20p13 which was confirmed by positional cloning method. The linkage analysis and genome wide screening of 260 families in the United Kingdom and United States found that this position has a putative candidate gene for the development of asthma and BHR.[11] Various population studies reported a range of SNPs in the ADAM33 gene with conflicting results.[12-14] Therefore, these studies indicate the diversity in the relationship of ADAM33 polymorphisms towards asthma and suggested that these variations may be due to different nationalities.

ADAM33 is a putative candidate gene for the development of asthma. Thus, to confirm the association of ADAM33 polymorphisms towards asthma and to examine the role of various phenotypic characteristics, two SNPs (F+1G>A position in intron 6, rs511898 and V4C>G position in 3'UTR, rs2787094) were genotyped in a North Indian population.

MATERIALS AND METHODS

Ethical clearance

Ethical clearance was granted by the Ethics Committee, PGIMER, Chandigarh, India, for conducting this research

work *vide* approval memo no. PG-1Trg-10 on 21.9.2010 for conducting the research work on human blood samples. After doctor's diagnosis and fulfilling Global Initiative for Asthma (GINA) guidelines,[15] each patient were provided with written information about the study and a due consent was taken from each patient prior to inducting him/her in the study.

Inclusion/Exclusion criteria

Asthma patients for this study were recruited from different states of North India. A total of 481 patients enrolled as cases visiting OPD (Out Patient Department), Pulmonary Medicine at PGIMER, Chandigarh, and 483 age-matched, healthy individuals, without any symptoms of atopic, pulmonary disease, any other co-morbid disease or smoking habits were recruited as controls.

Lung function test

Spirometry tests were performed according to Association of Respiratory Technician and Physiologists (ARTP) guidelines[16] for generating pneumotachographs, in asthmatics using Spiro 233 (PK Morgan, Rainham, Kent, UK). Out of 481 asthma patients, 377 asthmatics have their spirometry data. The frequency of mild obstruction and heterozygous alleles in both polymerphisms were found to be higher in the studied population (Table 1).

Total IgE measurement

Total IgE was measured using ImmunoCAPS with device Phadia 100 IDM version 5.43 (Thermo Fisher Scientific Inc., Waltham, MA, USA) in serum samples of both control and asthma patients to screen allergy. Skin Prick Test (SPT) and serum specific IgE against *Aspergillus fumigatus* were also done in some patients to distinguish asthma and ABPA (Allergic bronchopulmonary aspergillosis). Only negative SPT patients with specific IgE < 0.35 KUA/L were recruited in the study.

Blood sample collection

Approximately 5ml blood was collected from each patient as well as control subjects in EDTA coated vials and stored at -80^{o}C until genomic DNA extraction. DNA isolation was done from frozen whole blood samples using SSC Buffer method[17] and checked on 0.8% agarose gel before storage at -20^{o}C for further use.

Genotyping

The amplification of the ADAM33 F+1G>A and V4C>G polymorphisms were done using PCR-RFLP method[18]. The specific primers for the F+1 G>A (rs511898) position in intron 6, were (F) 5'-GTATCTATAGCC-CTCCAAATCAGAAGAGCC-3' and (R) 5'-GGACCC-TGAGTGGAAGCTG-3'. For the V4C>G (rs2787094) position in 3'UTR, (F)5'-CTCAGGAACCACCTAGG-GGAGAAG-3' and (R)5'-CAAAGGTCACACAGCCC-CTGA CCT-3'.

The PCR was carried out in a thermal cycler, in a total volume of 25µl containing: 10X PCR Buffer, 3 mM $MgCl_2$, 1 mg/ml nuclease free BSA , 50 pmol of each primer, either primer for R1/R2 alleles, 10 mM of each dNTP, 0.125 U Taq polymerase and 2µl genomic DNA in the conditions: 95°C for 5 min, followed by 40 cycles of 95°C for 45s, 60-65°C for 45s, 72°C for 30s and 72°C for 10 min for final extension.

Identification of ADAM33 F+1G>A (rs511898) and V4C>G (rs2787094) polymorphisms

For F+1G>A polymorphism, 10µL of the PCR product was digested overnight at 37ºC with 5U of restriction endonuclease, *MspI*. After digestion, an uncut 166 bp indicated the mutant type AA genotype. The heterozygote GA genotype was indicated by bands at 166 bp, 137 bp and 29 bp while the wild type GG genotype was indicated by bands at 137 bp and 29 bp (Figure 1).

For ADAM33 V4C>G polymorphism, 10µL of the PCR product was digested overnight at 37ºC with 5U of restriction endonuclease, *PstI*. After digestion, an uncut 290 bp indicated the wild type CC genotype. The heterozygote CG genotype was indicated by bands at 290 bp, 196 bp and 94 bp while the mutant type GG genotype was indicated by bands at 196 bp and 94 bp (Figure 2).

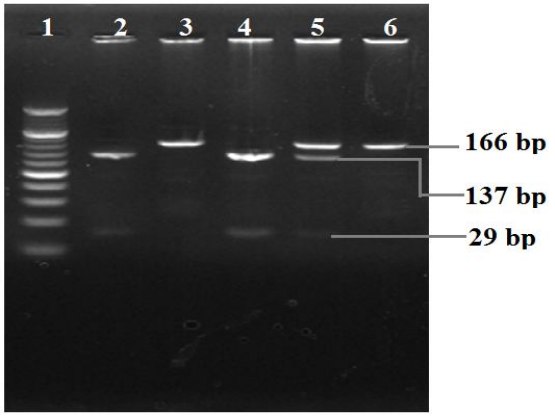

Figure 1. Restriction digestion (*MspI*) products of ADAM33 F+1G>A polymorphism on 3% agarose gel. Lane 1: 20 bp ladder. Lanes 2, 4: homozygous wild GG (137 bp and 29 bp). Lanes 3, 6: homozygous mutant AA (166 bp). Lane 5: heterozygous GA (166 bp, 137 bp and 29 bp).

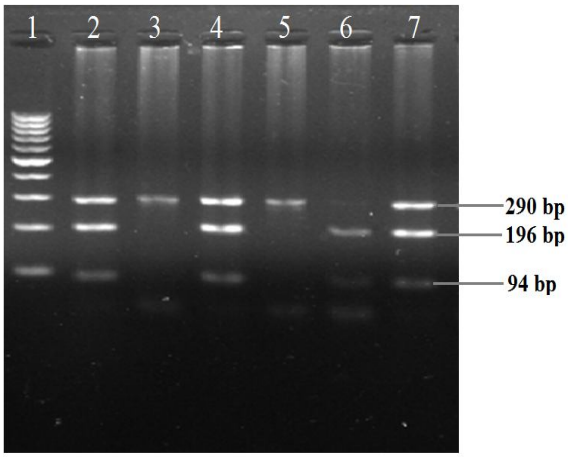

Figure 2. Restriction digestion (*PstI*) products of ADAM33 V4C>G polymorphism on 2% agarose gel. Lane 1: 100 bp ladder. Lanes 2, 4, 7: heterozygous CG (290 bp, 196 bp and 94 bp). Lanes 3, 5: homozygous wild CC (290 bp). Lane 6: homozygous mutant GG (196 bp and 94 bp).

Statistical analysis

All the statistical analyses were performed using the SPSS software for Windows version 20.0 (SPSS, Inc., Chicago, IL, USA) and Epi Info version 3.4.7 (Centers for Disease

Control and Prevention, Atlanta, GA, USA). Chi-square analysis was used to check the deviation from Hardy-Weinberg equilibrium (HWE) and to compare the genotype and allele frequency between asthma and control groups. Odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors and p value < 0.05 was considered as statistically significant.

RESULTS

Demographic characteristics

In this study, ADAM33 F+1G>A (rs511898) and V4C>G (rs2787094) polymorphisms were genotyped in a total of 964 subjects, including 483 healthy controls and 481 asthma patients. Various characteristics such as gender, age, disease duration, atopic status, IgE level, smoke exposure, family history, cough, severity, BMI *etc*. were examined. The mean age for asthma patients was found to be 37 years and for healthy adults was 34 years (Table 1).

Table 1. Characteristics of studied population.

Phenotypic traits	Asthma patients n (%)	Controls n (%)
Gender	481	483
Males	191 (39.7)	189 (39.1)
Females	290 (60.3)	294 (60.9)
Age (Mean ± SD)	37.22 ± 14.1	34.29 ± 12.2
Disease duration (years)	9.23	0
Rhinitis		
Allergic rhinitis	397 (82.5)	0
No rhinitis	84 (17.5)	483
Allergy		
Allergic to at least 2 provoking factors	400 (83.5)	0
Non- allergic	81 (16.8)	483
Smoking status		
Ever-smoker	56 (11.6)	0
Non-smoker	425 (88.5)	483
Spirometry data[a]	(n = 377)	
FVC observed	2.94 ± 1.1	
FVC predicted	2.23 ± 1.6	
FEV1 observed	2.74 ± 1.1	ND
FEV1 predicted	2.61 ± 0.66	
FEV1/FVC observed (%)	68.92 ± 13.5	
FEV1/FVC predicted (%)	87.81 ± 47.1	
Family history (n%)	135 (28%)	0
Weight (kg)	59.6	60.54
Height (cm)	159.5	161.36
BSA (m^2)	1.61	1.59
BMI (kg/m^2)	23.7	23.96
Underweight ≤18.5	16.6	17.4
Normal weight = 18.5–24.9	21.15	21.3
Overweight = 25–29.9	26.85	26.7
Obesity ≥ 30	33.7	30.8
IgE (IU/ml)[b]	2651.7	776.1
Asthma severity[a]	n = 377	
Normal	126	
Mild obstruction	153	ND
Moderate obstruction	65	
Severe obstruction	33	

FVC – Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, BSA – Body Surface Area, BMI – Body Mass Index, ND – Not Done, % - frequency.

[a] Spirometry test was conducted for 377 asthma patients

[b] IgE levels were confirmed for 213 asthma patients and 125 controls and given as average in IU/ml.

Prevalence of allelic and genotypic frequencies in ADAM33 F+1G>A (rs511898) and V4C>G (rs2787094) polymorphisms

Both the polymorphisms in studied population follow Hardy-Weinberg Equilibrium (HWE). Statistical analysis of the allelic frequencies for F+1G>A position indicated a non-significant association towards the disease with p=0.970. However, in V4C>G the mutant G allele has increased trends among the asthma patients (42.3%) than in the controls (34.5%) having highly significant association towards the disease with OR=1.39, 95% CI (1.15-1.68) and p=0.000 (Table 2).

While comparing the genotype frequencies, the results revealed a non-significant p (>0.05) for F+1G>A polymorphism (Table 2). Statistical data for the genotype frequencies in V4C>G revealed that the homozygous wild CC genotype has increased trends among the controls (41.0%) than the asthma patients (31.4%). The heterozygous genotype CG has increased frequency among the asthma patients (52.6%) as compared to the controls (49.1%) with a highly significant OR=1.40, 95% CI (1.05-1.86) and p=0.017. However, the homozygous mutant GG genotype was slightly more prevalent in the asthmatics (16.0%) than in the control subjects (9.9%) with a significant OR=2.10, 95% CI (1.36-3.27) and p=0.000. The CG+GG genotypic combination again conferred risk towards asthma with OR=1.51, 95% CI (1.16-2.00) and p=0.002 (Table 2).

Phenotypic characteristics of ADAM33 F+1G>A (rs511898) and V4C>G (rs2787094) polymorphisms towards asthma

Further categorizing the asthma patients on the basis of the phenotypic characteristics of the disease (Table 3), as obtained from their detailed proforma, such as sex (male/ female), occurrence (seasonal/ throughout), severity (wheeze on exertion/ wheeze at rest), family history (positive/ nil), allergy to at least 2 provoking factors (positive/ nil), smoking status (non-smoker/ ever-smoker) and longstanding cough (positive/ nil), significant association was only found between F+1 polymorphism and asthma while comparing male sex with OR=0.68, 95% CI (0.51-0.92), p= 0.009 and female sex with OR=1.29, 95% CI (1.02-1.63), p= 0.031. However, no association was found between F+1 and other phenotypic characteristics towards asthma.

In addition, significant risk was found between V4 polymorphism and asthma while comparing sex (male/ female) with OR=1.59, 95% CI (1.17-2.15), p=0.002 in male and with OR=1.28, 95% CI (1.00-1.63), p=0.040 in female, occurrence (seasonal/ throughout) with OR=1.37, 95% CI (1.10-1.70), p=0.003 in seasonal occurrence and with OR=1.43, 95% CI (1.12-1.85), p=0.004 in throughout symptoms, wheeze on rest with OR=1.46, 95% CI (1.19-1.80), p=0.000, family history (positive/ nil) with OR=1.57, 95% CI (1.18-2.08) and p=0.001 having family history and with OR=1.33, 95% CI (1.08-1.64), p=0.005 in non family history, allergic (positive/ nil) with OR=1.37, 95% CI (1.12-1.67) and p=0.001 having allergy and with OR=1.52, 95% CI (1.07-2.16) and p=0.014 in non allergic, rhinitis with OR=1.45, 95% CI (1.19-1.76),

p=0.001, longstanding cough (positive/ nil) with OR=1.37, 95% CI (1.06-1.77) and p=0.012 having cough and with OR=1.41, 95% CI (1.14-1.74), p=0.001 having no cough, smoking status (non-smoker/ ever-smoker) with OR=1.37, 95% CI (1.13-1.66), p=0.001 in non-smoker and with OR=1.59, 95% CI (1.05-2.40), p=0.021 in ever-smoker respectively (Table 4).

Table 2. Genotypic and allelic frequencies of polymorphisms at ADAM33 F+1G>A (rs511898) and V4 C>G (rs2787094).

Polymorphisms	Asthma Patients 481 (%)	Controls 483 (%)	χ^2	OR (95% CI)	p
Genotypic frequencies					
ADAM33 F+1 (rs511898)					
GG	134 (27.9)	139 (28.8)		Ref (1.0)	
GA	232 (52.8)	247 (51.1)	0.18	1.07 (0.29 - 1.45)	0.668
AA	115 (19.3)	97 (20.1)	0.00	0.99 (0.67 - 1.47)	0.927
GA + AA	347 (72.1)	344 (71.2)	0.10	1.05 (0.78 - 1.40)	0.751
ADAM33 V4 (rs2787094)					
CC	151 (31.4)	198 (41.0)		Ref (1.0)	
CG	253 (52.6)	237 (49.1)	5.71	1.40 (1.05 - 1.86)	0.017*
GG	77 (16.0)	48 (9.9)	12.39	2.10 (1.36 - 3.27)	0.000*
CG + GG	330 (68.6)	285 (59.0)	9.62	1.51 (1.16 - 2.00)	0.002*
Allelic frequencies					
ADAM33 F+1 (rs511898)					
G	522 (54.3)	525 (54.3)		Ref (1.0)	
A	440 (45.7)	441 (45.7)	0.00	1.00 (0.84 - 1.21)	0.970
ADAM33 V4 (rs2787094)					
C	555 (57.7)	633 (65.5)		Ref (1.0)	
G	407 (42.3)	333 (34.5)	12.51	1.39 (1.15 - 1.68)	0.000*

Ref – reference, $\chi 2$ – chi square, OR – odds ratio, CI – confidence interval, % - frequency, * - significant value.

Table 3. Phenotypic characteristics and ADAM33 F+1G>A (rs511898) polymorphism.

Phenotypic traits	n (%)	AA n (%)	AG n (%)	GG n (%)	A n (%)	G n (%)	χ^2	OR (95% CI)	p
Controls	483	139 (28.8)	247 (51.1)	97 (20.1)	525 (54.3)	441 (45.7)		Ref (1.0)	
Males	189 (39.1)	47 (24.9)	92 (48.7)	50 (26.5)	186 (49.2)	192 (50.8)			
Females	294 (60.9)	92 (31.3)	155 (52.7)	47 (16.0)	339 (57.7)	249 (42.3)			
Asthmatics	481								
Sex									
Males	191 (39.7)	71 (37.2)	82 (42.9)	38 (19.9)	224 (58.6)	158 (41.4)	6.80	0.68 (0.51-0.92)	0.009*
Females	290 (60.3)	63 (21.7)	172 (59.3)	55 (19.0)	298 (51.4)	282 (48.6)	4.64	1.29 (1.02-1.63)	0.031*
Occurrence									
Seasonal	295 (61.3)	84 (28.5)	160 (54.2)	51 (17.3)	328 (55.6)	262 (44.4)	0.06	0.97 (0.79-1.20)	0.808
Throughout	186 (38.7)	50 (26.9)	94 (50.5)	42 (22.6)	194 (52.2)	178 (47.8)	0.52	1.09 (0.85-1.40)	0.470
Severity									
Wheeze on exertion	126 (26.2)	41 (32.5)	63 (50.0)	22 (17.5)	145 (57.5)	107 (42.5)	0.82	0.88 (0.66-1.17)	0.364
Wheeze at rest	355 (73.8)	93 (26.2)	191 (53.8)	71 (20.0)	377 (53.1)	333 (46.9)	0.26	1.05 (0.86-1.28)	0.612
Family history									
Family history (nil)	346 (71.9)	96 (27.7)	190 (54.9)	60 (17.3)	382 (55.2)	310 (44.8)	0.12	0.97 (0.79-1.18)	0.730
Family history (+ve)	135 (28.1)	38 (28.1)	64 (47.7)	33 (24.4)	140 (51.9)	130 (48.1)	0.53	1.11 (0.84-1.46)	0.467
Rhinitis									
Rhinitis (nil)	84 (17.5)	15 (17.9)	52 (61.9)	17 (20.2)	82 (48.8)	86 (51.2)	1.76	1.25 (0.89-1.76)	0.184
Rhinitis (+ve)	397 (82.5)	119 (30.0)	202 (50.9)	76 (19.1)	440 (55.4)	354 (44.6)	0.20	0.96 (0.79-1.16)	0.654
Allergy									
Allergy (nil)	81 (16.8)	23 (28.4)	44 (54.3)	14 (17.3)	90 (55.6)	72 (44.4)	0.08	0.95 (0.67-1.36)	0.775
Allergy (+ve)	400 (83.2)	111 (27.8)	210 (52.5)	79 (19.8)	432 (54.0)	368 (46.0)	0.02	1.01 (0.84-1.23)	0.884
Smoking status									
Non smoker	425 (88.4)	108 (25.4)	236 (55.5)	81 (19.1)	452 (53.2)	398 (46.8)	0.25	1.05 (0.87-1.27)	0.617
Ever smoker	56 (11.6)	26 (46.4)	18 (32.1)	12 (21.4)	70 (62.5)	42 (37.5)	2.70	0.71 (0.47-1.09)	0.100
Cough									
Cough (nil)	302 (62.8)	85 (28.1)	158 (52.3)	59 (19.5)	328 (54.3)	276 (45.7)	0.00	1.00 (0.81-1.24)	0.987
Longstanding cough	179 (37.2)	49 (27.4)	96 (53.6)	34 (19.0)	194 (54.2)	164 (45.8)	0.00	1.01 (0.78-1.29)	0.959

n – number of subjects, Ref – reference, $\chi 2$ – chi square, OR – odds ratio, CI – confidence interval, % - frequency, * - significant value.

Table 4. Phenotypic characteristics and ADAM33 V4C>G (rs2787094) polymorphism.

Phenotypic traits	n (%)	T/T n (%)	T/C n (%)	C/C n (%)	T n (%)	C n (%)	χ^2	OR (95% CI)	p
Controls	483	198 (41.0)	237 (49.1)	48 (9.9)	633 (65.5)	333 (34.5)		Ref (1.0)	
Males	189 (39.1)	78 (41.3)	96 (50.8)	15 (7.9)	252 (66.7)	126 (33.3)			
Females	294 (60.9)	120 (40.8)	141 (48.0)	33 (11.2)	381 (64.8)	207 (35.2)			
Asthma patients	481								
Sex									
Males	191 (39.7)	56 (29.3)	101 (52.9)	34 (17.8)	213 (55.8)	169 (44.2)	9.52	1.59 (1.17-2.15)	0.002*
Females	290 (60.3)	95 (32.8)	152 (52.4)	43 (14.8)	342 (59.0)	238 (41.0)	4.21	1.28 (1.00-1.63)	0.040*
Occurrence									
Seasonal	295 (61.3)	95 (32.2)	153 (51.9)	47 (15.9)	343 (58.1)	247 (41.9)	8.56	1.37 (1.10-1.70)	0.003*
Throughout	186 (38.7)	56 (30.1)	100 (53.8)	30 (16.1)	212 (57.0)	160 (43.0)	8.42	1.43 (1.12-1.85)	0.004*
Severity									
Wheeze on exertion	126 (26.2)	44 (34.9)	66 (52.4)	16 (12.7)	154 (61.1)	98 (38.9)	1.71	1.21 (0.90-1.63)	0.192
Wheeze at rest	355 (73.8)	107 (30.1)	187 (52.7)	61 (17.2)	401 (56.5)	309 (43.5)	14.18	1.46 (1.19-1.80)	0.000*
Family history									
Family history (nil)	346 (71.9)	115 (33.2)	177 (51.2)	54 (15.6)	407 (58.8)	285 (41.2)	7.77	1.33 (1.08-1.64)	0.005*
Family history (+ve)	135 (28.1)	36 (26.7)	76 (56.3)	23 (17.0)	148 (54.8)	122 (45.2)	10.41	1.57 (1.18-2.08)	0.001*
Rhinitis									
Rhinitis (nil)	84 (17.5)	28 (33.3)	48 (57.1)	8 (9.5)	104 (61.9)	64 (41.1)	0.83	1.17 (0.82-1.66)	0.364
Rhinitis (+ve)	397 (82.5)	123 (31.0)	205 (51.6)	69 (17.4)	451 (56.8)	343 (43.2)	14.03	1.45 (1.19-1.76)	0.001*
Allergy									
Allergy (nil)	81 (16.8)	21 (25.9)	48 (59.3)	12 (14.8)	90 (55.6)	72 (44.4)	6.00	1.52 (1.07-2.16)	0.014*
Allergy (+ve)	400 (83.2)	130 (32.5)	205 (51.2)	65 (16.2)	465 (58.1)	335 (41.9)	10.20	1.37 (1.12-1.67)	0.001*
Smoking status									
Non smoker	425 (88.4)	136 (32.0)	222 (52.2)	67 (15.8)	494 (58.1)	356 (41.9)	10.54	1.37 (1.13-1.66)	0.001*
Ever smoker	56 (11.6)	15 (26.8)	31 (55.4)	10 (17.9)	61 (54.5)	51 (45.5)	5.36	1.59 (1.05-2.40)	0.021*
Cough									
Cough (nil)	302 (62.8)	91 (30.1)	165 (54.2)	46 (15.2)	347 (57.5)	257 (42.5)	10.34	1.41 (1.14-1.74)	0.001*
Longstanding cough	179 (37.2)	60 (33.5)	88 (49.2)	31 (117.3)	208 (58.1)	150 (41.9)	6.22	1.37 (1.06-1.77)	0.012*

n – number of subjects, Ref – reference, $\chi 2$ – chi square, OR – odds ratio, CI – confidence interval, % - frequency, * - significant value.

DISCUSSION

The present study revealed a significant association between V4C>G and asthma in both the allelic (OR=1.39, p=0.000) and the genotypic frequencies of CG (OR=1.40, p=0.017) and GG (OR=2.10, p=0.000) (Table 2). Also, most of the phenotypic characteristics including sex, occurrence, wheeze, family history, allergy, rhinitis, cough, smokers and non-smokers showed significant risk towards the disease. However, protective association in male and risk in female was found between F+1G>A and asthma in the studied population. Total serum IgE level was also found to be highly elevated in asthmatics than the healthy controls. Spirometry data was only available for asthma patients so we are unable to apply the statistics (Table 1).

ADAM33 is the first putative asthma candidate gene as it has genetic association in BHR that accounts its involvement in asthma.[11] However, exact mechanism of ADAM33 in asthma is still unclear. Various studies found that the asthma is an inflammatory disease and soluble ADAM33 (sADAM33) promotes angiogenesis, which is a non-inflammatory response that increases the expression of angiogenic mediators and their receptors thereby reduced lung function.[19,20] In addition to this, change of the ADAM33 metalloproteases location, results in improper cleavage of angiogenic substrates that lead to increased angiogenesis in the airway wall followed by airway wall thickening, obstruction and reduced lung function in asthmatics. A high level of sADAM33 has also been reported in bronchoalveolar lavage fluid (BALF) of asthmatics, and is therefore predicted that they are involved in the disease severity and reduced lung function.[21] Although, ADAM33 is associated with non-inflammatory mechanisms of airway remodeling, it can also promote inflammation through altered ADAM33 protein function leading to enhanced release of cytokines and growth factors.[22] Lastly, the environment and the ADAM33 proteins interactions could be important in the pathogenesis of the disease.

Studies performed in different human populations have suggested a diverse role of ADAM33 SNPs in the development of the atopic diseases. In the present study, ADAM33 V4C>G polymorphism is significantly associated with asthma in a North Indian population. In agreement with our study, V4 polymorphism was reported to be associated with asthma in a Chinese population.[13,23] Significant association was also reported between ADAM33 and V4 with asthma in the American population (p=0.032).[5] Another study conducted on a total of 120 adults having allergic rhinitis and 128 normal healthy controls found to have significant association with allergic rhinitis among Jordanians.[24] Our findings are partly in accordance with the study conducted on different region from North India including 175 cases and 253 controls. They suggested both the (F+1 and V4)

polymorphisms were associated with increased risk for asthma.[18] This varied result may be due to difference in region, sample size and the population mixture.

In support of our findings for F+1 polymorphism, a case-control study including 296 asthma patients and 270 healthy controls found no significant association in the Chinese population.[12] Schedel *et al.* also conducted a study on German children of 9-11 years old, suggested that the F+1 polymorphism of the ADAM33 gene is not associated with asthma.[9] No association was also observed in American[5] and Australian population.[25] Another study conducted on a Chinese population found no significant difference between the asthmatics and the healthy subjects for ADAM33 F+1 polymorphism (p=0.917).[26]

Several epidemiological findings are not reproducible as there are number of variables including age, experimental scenario, region, sample bias, population size *etc.* interact with the gene function. Therefore, in contrast to our study, studies on ADAM33 V4 polymorphism in population of Korean, Australia, Thailand, Cartagena, Colombia, Egyptian were unable to find any association of this SNP with asthma.[25, 27-30] Another study in the Dutch PIAMA cohort found that the A allele of F+1 increased the risk to develop asthma[14] that is in accordance with the female gender in the present study. Although, a meta- analysis on European and Asian population demonstrate significant association ADAM33 F+1 and V4 polymorphisms with asthma[31], recent study found that this meta-analysis were not entirely credible as there are some mistakes in the genotypic data of these polymorphisms.[32]

In the present study, V4 polymorphism has highly significant association towards asthma in correlation with several clinical parameters that influence the disease such as male (OR=1.59, p=0.002), female (OR=1.28, p=0.040, seasonal occurrence (OR=1.37, p=0.003), throughout occurrence (OR=1.43, p=0.004), wheeze on rest (OR=1.46, p=0.000), family history (OR=1.57, p=0.001), no family history (OR=1.33, p=0.005), allergic (OR=1.37, p=0.001), non allergic (OR=1.52, p=0.014), rhinitis (OR=1.45, p=0.001), longstanding cough (OR=1.37, p=0.012), no cough (OR=1.41, p=0.001), non-smoker (OR=1.37, p=0.001) and in ever-smoker (OR=1.59, p=0.021) (Table 4). However in F+1 polymorphism, protective association was only found in male (OR=0.68, p= 0.009) and risk was observed in female (OR=1.29, p= 0.031) (Table 3).

The disease-associated SNPs of the ADAM33 gene exist within the coding, 3'UTRs as well as deep within introns.[11] Both the polymorphisms (F+1G>A, and V4C>G) in the present study causes amino acid change[33] but whether they modify the structure of the protein is unknown. It is not always single nucleotide changes that affect the function of the protein. The complex interplay of genetic and environmental factors, type of clinical asthma and co-morbid illness in the controls might be responsible for these contradictory results. Moreover, epigenetic changes alter the patterns of DNA methylation which ultimately bring changes in the ADAM33 gene expression, resulting in potentially adverse biological effects.[34] Thus, epigenetic mechanisms in the expression of ADAM33 gene need to be explored to evaluate its role in asthma pathogenesis.

CONCLUSION

In the present study, ADAM33 F+1G>A and V4C>G polymorphisms were genotyped and our data suggest that V4C>G polymorphism plays an important role in the pathogenesis of asthma in a North Indian population. However, additional studies are needed to elucidate the molecular mechanism of ADAM33 polymorphisms and the effect of gene-environment interaction in the asthma pathogenesis.

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